Here we present data on liver metal levels and expression of genes related to iron homeostasis in rhesus monkeys after inhalational manganese exposure. Archived liver samples from rhesus monkeys exposed to 0 (\(n = 6\)), 0.06 (\(n = 6\)), 0.3 (\(n = 4\)) and 1.5 (\(n = 4\)) mg/m\(^3\) manganese inhalation for 65 days were obtained from a published study ("Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation" [1]). Samples were analyzed by spectroscopy, immunoblotting and quantitative PCR to assess metal levels and gene expression. Liver manganese and iron levels were linearly correlated although only the intermediate manganese exposure level (0.3 mg Mn/m\(^3\)) led to a statistically significant increase in liver iron levels.

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More specific subject area

Type of data: Figures and tables

How data was acquired: Spectroscopy; immunoblotting; quantitative PCR (see materials and methods for instrument specifics)

Data format: Analyzed

Experimental factors: Archived liver samples from monkeys subjected to inhalational manganese exposure

Experimental features: Archived monkey liver samples were analyzed by spectroscopy, immunoblotting and quantitative PCR to assess metal levels and gene expression.

Data source location: Not applicable

Data accessibility: Data is within this article

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**Fig. 1.** Liver metal levels in rhesus monkeys after manganese inhalation. (A–D) Levels of manganese (Mn, A), iron (Fe, B), copper (Cu, C) and zinc (Zn, D) were measured by ICP-AES and GF-AAS in livers from monkeys subjected to 0–1.5 mg/m³ manganese inhalation for 65 days. Circles represent average values from 4–6 livers; bars indicate standard deviation. * Indicates statistical significance (*p* < 0.05) compared to control group (0 mg/m³) as calculated by one-way ANOVA with Dunnett’s method. (E and F) Liver Mn vs. Fe levels (E) and Cu vs. Zn levels (F) were plotted along with rho (ρ) and *p* values as calculated by Spearman’s rank correlation test.
Value of the data

- Inhalational manganese exposure perturbed liver iron levels and expression of BMP6, a known regulator of systemic iron homeostasis.
- While sample size in this study is small, the potential value of this data is that it includes an analysis of factors involved in both cellular and systemic iron homeostasis.
- Our analysis may be useful as a reference for future, larger studies.

1. Data

We analyzed levels of manganese, iron, copper and zinc and expression of genes involved in iron homeostasis in archived liver samples from rhesus monkeys after inhalational manganese exposure [1]. Fig. 1 depicts liver levels of manganese, iron, copper and zinc. Figs. 2–5 depict levels of various

![Liver ferritin levels in rhesus monkeys after manganese inhalation.](image)

**Fig. 2.** Liver ferritin levels in rhesus monkeys after manganese inhalation. (A) Levels of ferritin heavy chain (FTH1) and light chain (FTL) and GAPDH were measured by immunoblot in protein lysates extracted from livers of monkeys after manganese inhalation. (B and C) Levels of immunoblot signal for FTH1 (B) and FTL (C) were measured relative to GAPDH signal by densitometry and plotted vs. exposure level. * Indicates statistical significance (p < 0.05) compared to control group (0 mg/m³) as calculated by one-way ANOVA with Dunnett's method. (D) Liver FTH1:GAPDH ratios were plotted against liver iron (Fe) levels and FTL:GAPDH levels along with rho (ρ) and p values as calculated by Spearman's rank correlation test.
factors involved in iron storage, export, import and regulation of gene expression. Specifically, Fig. 2 depicts levels of ferritin, an intracellular iron storage protein abundantly expressed in conditions of iron excess. Fig. 3 depicts levels of factors that regulate cellular iron excess: BMP6, a protein abundantly expressed in conditions of iron excess; HAMP, a liver-derived hormone also known as hepcidin that negatively regulates iron excess and is stimulated by BMP6; SLC40A1, a cellular iron export protein also known as ferroportin that is negatively regulated by HAMP. Fig. 4 depicts levels of
transferrin receptor, an iron import protein that mediates cellular uptake of iron bound to the serum protein transferrin. Fig. 5 depicts levels of iron regulatory protein 2, an RNA-binding protein that regulates expression of genes such as ferritin and transferrin receptor in response to changes in cellular iron levels. Table 1 presents analysis of correlations between all measured parameters in Figs 1–5. Table 2 presents hematologic data previously referenced as data not shown in the original study [1].

2. Experimental design, materials and methods

2.1. Animals

Archived samples of rhesus monkey liver were obtained from a previously published study [1]. Details concerning the animals, their husbandry and manganese inhalation have been published [1]. Male rhesus monkeys purchased from Covance Research Products, Inc. (Alice, TX) were used.
Monkeys were approximately 20–24 months old at the start of the inhalation exposure. Monkeys were exposed to MnSO₄ for 6 h/day, 5 days/week, for 13 weeks (65 exposure days). These monkeys were allocated as follows: 0.0 (n=6), 0.06 (n=6), 0.3 (n=4), and 1.5 (n=4) mg Mn/m³. Food was withheld overnight prior to necropsy. Monkeys were anesthetized with ketamine (20 mg/kg, i.m., Fort Dodge Animal Health, Fort Dodge, IA), blood collected, and then euthanized with pentobarbital (80–150 mg/kg, i.v., Henry Schein, Inc., Port Washington, NY) followed by exsanguination. Liver samples were stored in individual plastic vials or bags, frozen in liquid nitrogen, and stored at approximately −80 °C until analyses were performed.

2.2. Tissue analyses

Frozen liver samples (50–100 mg) were thawed and digested in 1 mL trace metal grade nitric acid (Fisher) at 65 °C for 2 h, then diluted 25-fold with MilliQ-purified water (Millipore). Digested samples were analyzed for manganese and iron concentrations in triplicate by inductively coupled plasma
atomic emission spectrometry (ICP-AES) using a JY2000 Ultrace spectrometer (Horiba) or by graphite furnace atomic absorption spectrometry (GF-AAS) using an AAnalyst 600 spectrometer (Perkin Elmer) in the Environmental Chemistry Facility at Brown University using previously described protocols[2]. Reference standards were analyzed repeatedly during each run to ensure run consistency. Additional samples of liver were used to assess biochemical endpoints. RNA was extracted from thawed liver tissue and analyzed by quantitative polymerase chain reaction (QPCR) using Taqman assays (Life Technologies).

Table 1
Spearman’s rank correlations between parameters of iron homeostasis in monkeys exposed to inhalation of 0–1.5 mg Mn/m³ over 65 days.

| Parameters include: liver manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) levels (µg/g liver), liver RNA and protein levels (relative to GAPDH RNA and protein levels respectively; arbitrary units), plasma Fe levels (µg/dL), plasma transferrin (TF) levels (mg/dL), total iron binding capacity (TIBC; mg/dL), TF saturation (Tf sat; %), red blood cell count (RBC; ×10⁶/µL), hemoglobin (HGB; g/dL), hematocrit (HCT; %), mean corpuscular volume (MCV; IL), mean corpuscular hemoglobin (MCH; pg); mean corpuscular hemoglobin concentration (MCHC; %). |
Densitometry of immunoblots was performed using ImageJ[3].

2.3. Statistics

Following an assessment of normality by Shapiro–Wilk test, the data for continuous variables were inter-compared for the exposure groups by analysis of variance (ANOVA). If the exposure’s main effect was significant, a Dunnett’s test was used to compare the three MnSO₄ exposure levels to the air-exposed controls. Unless otherwise noted, data presented are for the mean values ± standard deviation. Correlations between measured parameters were assessed by Spearman Rank Order Correlation test. A probability value of $p=0.05$ was used as the critical level of significance for all statistical tests. Statistical analysis was performed using Sigmaplot (Systat Software). Analysis did not take into account effect of multiple comparisons on calculation of statistical significance.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.01.055.
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