Early-life iron excess causes iron overload and enhances purine degradation via activation of xanthine oxidase in developing hippocampus in a nursing piglet model

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Abstract: 224

INTRODUCTION & HYPOTHESIS

- In many developed countries, prophylactic iron (supplements and fortified formula) is commonly given to infants older than 4–6 months without a screening test for iron status.
- Recent study showed that iron regulatory mechanism (hepcidin – ferroportin axis) is not functionally mature in suckling mouse pups (Frazer et al., 2017).
- There is a growing concern over the neurological effects of dietary iron overexposure in iron-replete infants (Hare et al., 2018).
- Nursing piglet is a promising preclinical model to investigate the impact of iron overload on neurodevelopment and cognitive function in infants.
- We hypothesize that iron over-supplementation causes iron overload, alters hippocampal metabolome and affects sociability in nursing piglets

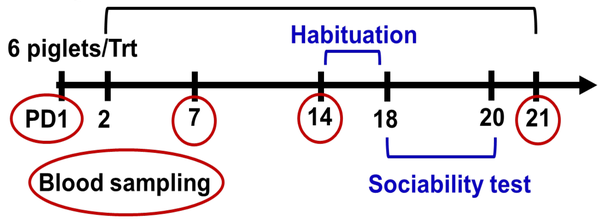
MATERIALS & METHODS

- EXPERIMENTAL DESIGN:

Twenty-four newborn piglets were randomly assigned to treatments from postnatal day 2 to 21. Piglets were nursed by sow throughout the study.

Treatments:

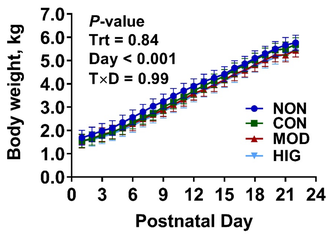
- CON: i.m. injection iron dextran on PD2 (100 mg iron)
- Ferrous sulfate drops:
  - NON: No iron (saline solution)
  - MOD: Moderate iron, 10 mg/(kg BW·d)
  - HIG: High iron, 50 mg/(kg BW·d)

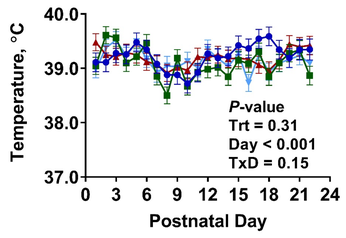


- ANALYSES
  - ✓ Tissue iron: Atomic absorption spectrometry
  - ✓ Non-targeted metabolomics (HIG vs. NON): GC-TOF MS for primary metabolites (West Coast Metabolomics Center, NIH)
  - ✓ Gene and protein expression: RT-qPCR and western blot
  - ✓ Activity of xanthine oxidase: ELISA (Cayman Chemical)
  - ✓ Behavioral analysis: EthoVision XT (Noldus)

RESULTS

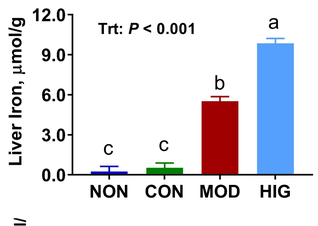
- Excess iron did not affect growth nor cause infection in nursing pigs.

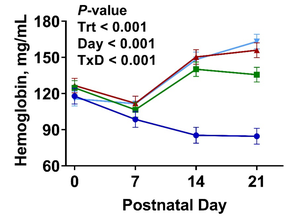


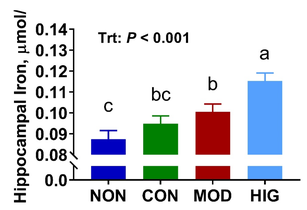


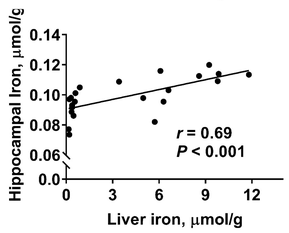
RESULTS

- Excess iron resulted in iron overload in the liver and hippocampus.
- The hippocampal iron content positively correlated with the hepatic iron.

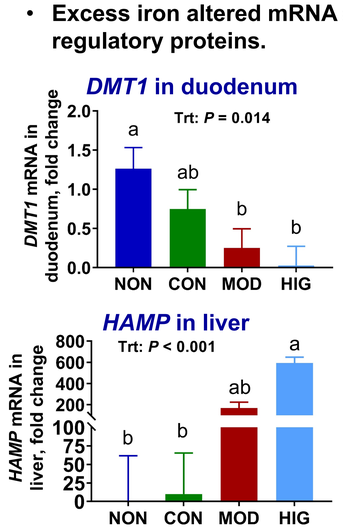


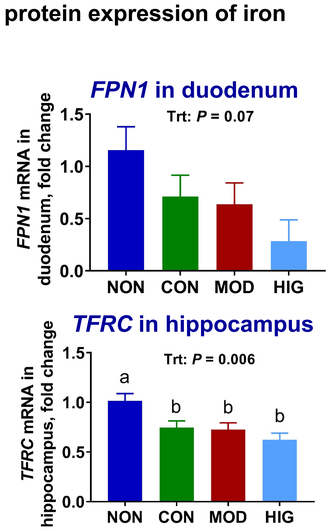


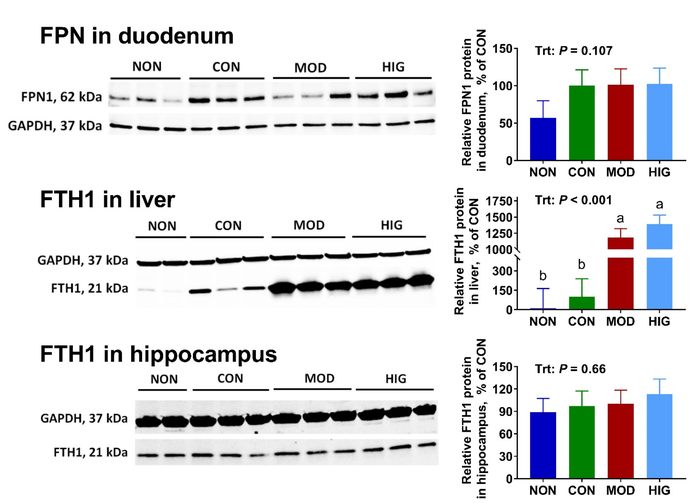




- Excess iron altered mRNA and protein expression of iron regulatory proteins.

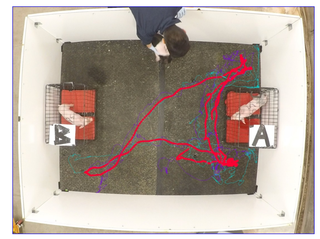


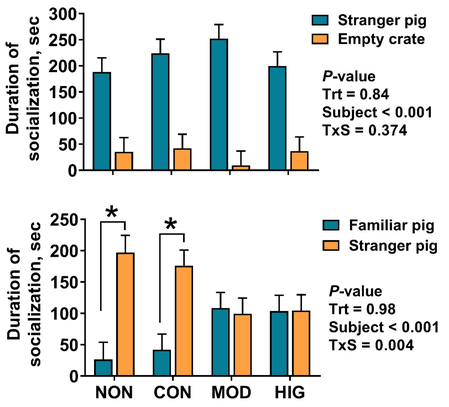




RESULTS

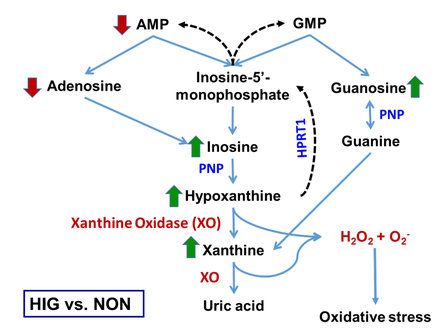
- Excess iron diminished socialization with novel conspecifics

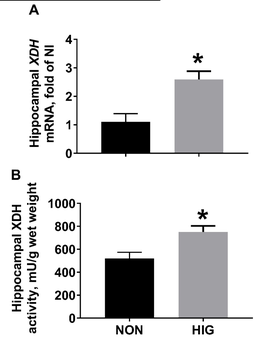


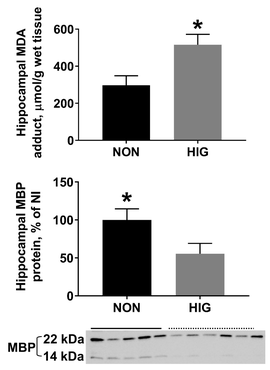


- Excess iron increased purine degradation and enhanced mRNA and activity of xanthine oxidase in the hippocampus (q < 0.2).

Metabolite	Fold change (HIG/NON)[1]	raw *P*-value	q-value[2]
Myo-inositol	0.86	0.001	0.01
Adenosine-5-monophosphate	0.19	0.002	0.09
Hypoxanthine	1.34	0.003	0.09
Inosine	1.36	0.004	0.09
Nicotinamide	0.81	0.006	0.10
Glycerol	1.27	0.008	0.10
Glutamine	2.36	0.009	0.10
Guanosine	3.23	0.010	0.10
N-acetylaspartic acid	0.84	0.014	0.14
Xanthine	2.34	0.019	0.16
Adenosine	0.50	0.020	0.16
Beta-alanine	1.83	0.023	0.16
Heptadecanoic acid	1.31	0.028	0.19
Stearic acid	1.15	0.032	0.19
Pantothenic acid	0.52	0.033	0.19







CONCLUSIONS

- Dietary Iron overexposure caused iron overload in the liver and hippocampus in a nursing pig model.
- Duodenal ferroportin expression was unresponsive to the increase of hepcidin.
- Hippocampal iron overload shifted purine flux towards degradation via activation of xanthine oxidase, which may contribute to the increased of lipid peroxidation.
- Iron overload impaired myelination in the hippocampus and diminished interaction with social novelty