Reviewer's report

Title: Psychological stress decreased iron absorption in rats

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This is an interesting paper regarding the effect of psychological stress on intestinal iron absorption. It involves providing rats 4-5 weeks of age (assumed given the weight) with bursts of electrical shock at 30 sec intervals. I assume ethical approval has been given for this protocol as I cannot see a statement to this effect.

The results suggest that although dietary intake and therefore iron absorption was the same, fecal iron absorption decreased with the length of PS and that this was ferroportin/hepcidin dependent. Although this is the conclusion no data is included here regarding hepcidin although a previous study indicates this relationship.

Major issues with this study that need to be resolved:

Hepatic hepcidin expression needs to be included on these rats to confirm the temporal pattern of iron absorption.

Spleen iron and ferroportin expression should be presented to demonstrate the hypoferrremia is not due to retention of iron by macrophages. It is likely macrophages contribute significantly to the hypoferrremia measured and be the more potent factor than intestinal iron absorption.

Importantly, the nature of the intestinal iron measurements are indirect rather than direct such as using isotopic measurements, the preferred method. Moreover the experimental design also may be problematic, that is PS is likely to increase intestinal transit. The fact that defecation was a response to PS indicates loss of control of bowel movements. Since non haem iron absorption is confined to the duodenum any rapid/uncontrolled gastric emptying will make iron absorption inefficient and this may account for the decreased iron absorption measured. This can be estimated by the period of the red coloration appearing in the feaces (not included) but still this is not as good as measuring isotopic iron absorption in duodenal situ gut sacs which is direct.

The way in which the protein was harvested from the duodenum appears suboptimal. The lack of protease inhibitors in the buffers used to recover protein from the duodenum is fraught with problems as the duodenum contains high levels of pancreatic derived proteases which can rapidly degrade the protein.

In summary although interesting the data is not convincing in its demonstration of
PS affects on iron absorption via hepcidin/ferroportin.