Long-term intermittent fasting induces changes to glucose metabolism and decreases apoptosis in the SAMP8 aged murine jejunum

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Objectives

The aim of this study was to determine the potential for intermittent fasting (IF) to ameliorate inherent deleterious age-related changes in the mammalian small intestine by assessing IF induced changes to gross structure, absorptive capability, apoptotic activity, and oxidative damage repair mechanisms in an age-accelerated murine model.

Background

- Intermittent fasting (IF) is a dietary practice involving cyclical time-restricted feeding that has gained popularity for its ease of adherence compared to other fasting methods and for the documented anti-inflammatory effect it has on the gastrointestinal tract[1,2].
- Previous research has shown that there are inherent-age related increases to murine jejunal crypt depth and villi length, attributed to decreased stem cell functioning, limiting homeostatic tissue maintenance[4]. Our lab has reproduced these morphological findings in aged mice and demonstrated that aging induces a decrease in jejunal wall thickness and an increase in caspase 3 expression[3].
- The opposing physiological effects of aging and fasting on the small intestine warrants further investigation into the interplay between the two in animal models. Improved understanding of this subject may allow for the development of specific IF regimens to be utilized therapeutically in the treatment of age-related small GI dysfunction.

Methods

Group	Feeding Protocol
1	Male ad-libitum (AL)
2	Male alternate day fasted (IF)
3	Female ad-libitum (AL)
4	Female alternate day fasted (IF)

Euthanize

- Serum/Blood
- Jejunum: section, H&E stain
- Jejunum: whole homogenate for western blot or PCR, or assay

- Two-month-old male and female SAMP8 mice were randomly assigned to the fasting group (IF) or ad-libitum control group (AL) (n=15/group). IF mice were fasted every other day for 9 months.
- Mice were euthanized per IACUC protocol #4138, and tissues were removed and either fixed in paraffin for sectioning or frozen at –80C for analysis via western blot/other assays.
- 5-micron jejunum sections were stained with H&E and morphological analysis of tissue was performed using light microscopy and quantified using Image J software.
- All data are represented as mean ± SEM
- * denotes statistical significance between AL and IF groups of the same gender, # denotes significant treatment effect between male and female fasting groups, P <0.05

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Results

Figure 1: Effect of Intermittent Fasting on Body Weight & Serum Glucose

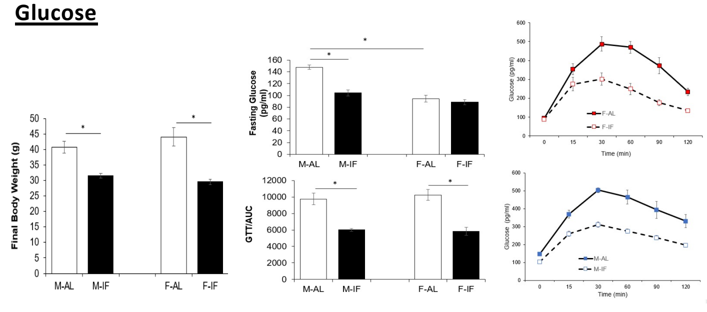

Figure 1. Body weight measurements and glucose tolerance testing in response to fasting. **Left:** Fasted mice gained significantly less weight over the course of the study, in both males (22% less, n=7-9, P<0.05) and females (33% less, n=7-9, P<0.05). **Middle & Right:** Lower fasting blood glucose levels and improved glucose tolerance testing were observed in both male and female fasted mice versus non-fasted.

Figure 2: Effect of Intermittent Fasting on Intestinal Morphology

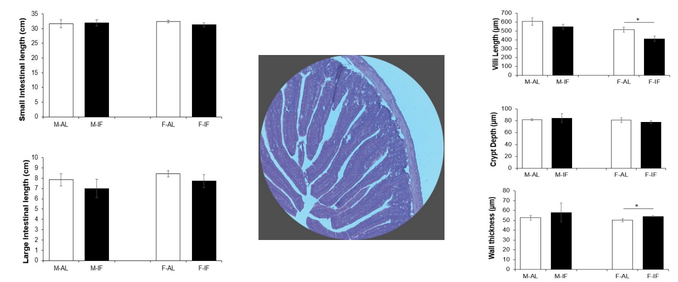

Figure 2. Morphological analysis of intestinal tissue samples. **Left:** No significant changes to average large or small intestinal gross length due to fasting were observed. **Right:** IF induced a significant decrease in average villi length and an increase in wall thickness in female mice compared to their non-fasted counterparts.

Figure 3: Effect of Intermittent Fasting on Intestinal Transporters

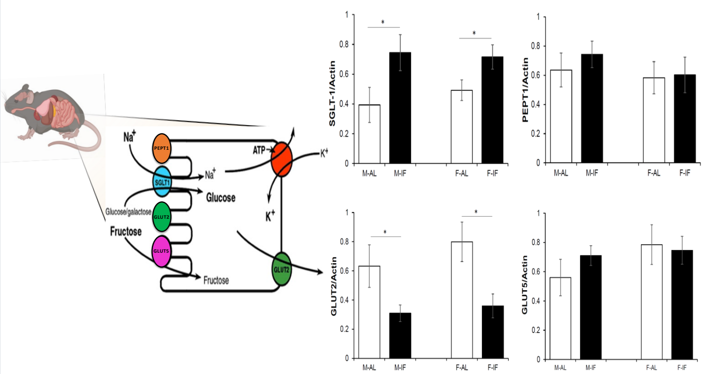

Figure 3. Western blot analysis of GLUT2, GLUT5, PEPT1, and SGLT1 expression in IF vs AL female and male mice. SGLT-1 expression was significantly increased in both IF males (by 1.92-fold) and IF females (by 1.46-fold), whereas GLUT2 expression was significantly decreased in both IF males (by 52%) and IF females (by 56%) compared to their non-fasted counterparts.

Figure 4: Effect of Intermittent Fasting on Oxidative Stress & Apoptotic Activity

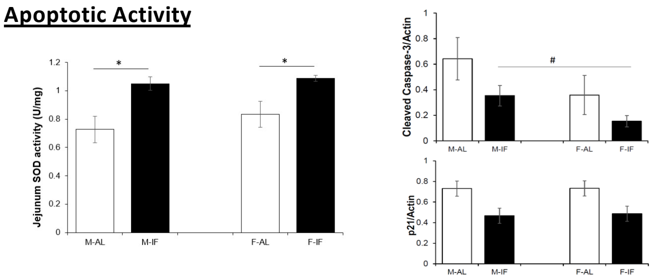

Figure 4. Left: SOD activity was significantly increased in both fasted groups compared to their freely fed counterparts, indicating that fasting may help prevent tissue damage due to increased oxidative stress over time. **Right:** Expression of cleaved Caspase-3 (top) and p21 (bottom) were both decreased in fasting mice. This may indicate that a fasted, less damaged jejunum has a decreased need to initiate cell cycle arrest by p21 expression, which may in turn have downstream effects in limiting the induction of apoptosis by Caspase-3.

Conclusion

Our data show that IF can be beneficial to intestinal health by decreasing intestinal villi length and increasing wall thickness while helping to prevent overall weight gain and increasing glucose tolerance. Fasting decreased GLUT2 expression while increasing SGLT-1 expression, which likely plays a role in altering glucose metabolism. Current work aims to identify the metabolomic pathways involved that may help explain the changes in SGLT-1 and GLUT2 expression. IF also contributes to gut health by increasing jejunal capacity to neutralize ROS while limiting apoptotic activity, which may partially explain fasting's documented anti-inflammatory effect. Overall, these findings indicate that certain IF regimens can have beneficial effects at the tissue level in both males and females.

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