SPECIFIC AIMS

It is well-established that long term calorie restriction (CR) has many beneficial effects, however, research has shown that adherence to long term CR is low. Alternate day fasting (ADF) is a diet regimen that has emerged as a possibly more viable alternative to traditional CR models because subjects are not required to restrict calories every day. Studies have shown ADF to have many of the same physiologic benefits as CR, including the improvements of biomarkers of some chronic diseases.

The small intestine is one of the first organs exposed to digested nutrients and is lined with a monolayer of intestinal epithelial cells of which are highly renewable. Because the intestinal epithelium is vital in the digestion and absorption of nutrients, we are interested in understanding how it is impacted by the recently popularized, ADF diet regimen. The following aims will test a central hypothesis that ADF will have similar protective effects as CR in metabolic and intestinal parameters.

AIM 1 will assess the effects of ADF on overall metabolic markers and phenotype. CD-1 mice will be randomly divided into one of two groups: ADF, fed ad libitum for 24 hours followed by removal of food for the next 24 hours, or control, fed ad libitum. The designated diet will be administered for 20 weeks. During the diet, food intake will be measured by weighing the amount of food remaining in the cage after feeding. After 20 weeks, body weight, fat mass, and plasma triglyceride levels will be measured. We hypothesize ADF mice will adapt to ADF by decreasing body weight, fat mass, and plasma triglycerides.

AIM 2 will assess the effects of ADF on the intestinal epithelium. Fasting and refeeding studies show the rapid effect of nutrients on crypt-villus morphology. Therefore, we will measure
crypt depth, villus height, and crypt cell number in ADF and control animals. **We hypothesize**

ADF will impact the crypt-villus axis by decreasing villus height and crypt depth.

Proliferation of the intestinal epithelium will be analyzed. To do this, the number of proliferating cells and mRNAs of genes implicated in pathways known to impact intestinal proliferation will be measured. **We hypothesize there will be a decrease in cell proliferation associated with decreased mRNAs involved in intestinal proliferation.**
Calorie restriction has been shown to have many physiologic benefits that can reduce chronic disease risk. A possibly more feasible alternative to the calorie restriction model is the alternate day fasting (ADF) regimen, consisting of giving food *ad libitum* for 24 hours followed by withholding food for 24 hours. ADF has also been shown to decrease cell proliferation in several different tissues. Currently, the effect of ADF on the intestine, the initial site of nutrient absorption, is unknown. We hypothesized that ADF would decrease proliferation in the intestinal epithelium leading to changes in gene expression associated with changes in intestinal growth and metabolism. CD-1 mice were fed standard chow *ad libitum* or ADF for 20 weeks. Body weight did not differ between groups, however, ADF mice displayed 60% lower gonadal fat mass and no detectable mesenteric fat compared to controls. There were no significant changes in villus height between groups, however, there was a 12% decrease in crypt depth associated with 13% fewer total cells per crypt in ADF mice compared to controls. Proliferation assessed by EdU (S-phase) was decreased by 20% in ADF mice compared to controls. To analyze ADF impact on the IGF1/insulin pathway, which promotes intestinal proliferation, mRNA levels of *Igf1, Igf1r, Ir, Gcg,* and *Ccnd1* were measured. A trend for decrease of mRNA levels was found for all genes in the jejunum, while significant decreases of *Igf1, Igf1r, Ir,* and *Ccnd1* were found in the colon. Measurements of mRNA levels for the insulin receptor (IR) isoforms, IR-A and IR-B, showed significantly increased IR-B:IR-A ratio in the jejunum and colon. Taken together, our data suggest despite similar caloric intake, ADF mice may limit intestinal proliferation by up-regulating IR-B expression to maximize intestinal nutrient absorption and protect against tumorigenesis.
INTRODUCTION

Calorie restriction (CR), a form of dietary restriction where caloric intake is reduced by 15-40% without risk of malnutrition, has been shown to reduce risk of chronic disease and demonstrate life-extending properties. Both animal and human studies have demonstrated it improves insulin sensitivity,\(^1,2\) cardiovascular health,\(^3-5\) and protects against oxidative stress.\(^6-9\) However, a more recently studied and popularized diet regimen, alternate day fasting (ADF), is thought to be a more feasible alternative to CR. In animal studies, ADF diet regimens have shown similar physiological benefits to those seen in CR in improving biomarkers of type 2 diabetes,\(^10-12\) cardiovascular disease,\(^12,14\) and cancer.\(^12,15-17\)

ADF regimens consist of giving food \textit{ad libitum} for 24 hours, followed by no food or reduced food intake during the next 24 hours. This pattern is then continuously repeated. A significant difference between ADF and CR is that overall caloric intake is typically not reduced in the ADF regimen, as mice tend to gorge on food during feed days. During the 24 hour \textit{ad libitum} feeding in the ADF diet, food intake is significantly increased to the extent that overall caloric intake of the ADF subject is equivalent to that of a control during the same length of time. Therefore, the significance of the ADF diet regimen is the pattern in which the food is consumed, not the amount. Although ADF has been less studied than CR, the ADF diet regimen has also been shown to produce many physiological benefits that play a role in reducing chronic disease risk. Decreases in circulating glucose concentrations have been reported in animals after 20-24 weeks on the ADF diet regimen,\(^18,19\) with one of the studies finding a reduction of glucose and insulin levels in the ADF treatment groups similar to the reduction seen in 40% CR treatment groups.\(^18\) Improved insulin sensitivity in humans has also been shown, after 2 weeks of ADF intervention.\(^20\) Animals had reduced heart rate and blood pressure following 16 weeks of an ADF
diet regimen, which again was similar to the reduction shown in 40% CR treatment groups.\textsuperscript{21} The cardiovascular benefits have been less conclusive in humans, however, one study found that after 3 weeks of ADF treatment, human subjects had increased circulating HDL cholesterol and decreased circulating TAG.\textsuperscript{22} The relationship between ADF and cancer risk factors have only been studied in animals, but many physiological changes associated with ADF have shown protective effects against cancer.\textsuperscript{23-25} Although many physiologic changes have been observed after ADF dietary interventions, the mechanisms in which these changes occur is not well defined.

Another effect that has been observed after treatment with ADF is decreased cell proliferation. In the study performed by Varady et al, mice had decreased proliferation of epidermal, splenic T, and mammary epithelial cells after a 4 week ADF diet regimen.\textsuperscript{26} Similar results were replicated in another study, which showed reduced proliferation of epidermal, prostate, liver, and splenic T cells.\textsuperscript{27} To analyze cell proliferation in both studies, deuterium label enrichment in DNA isolated from the different cell types was measured. Effects of ADF on overall intestinal physiology or intestinal epithelial proliferation have yet to be explored.

Upon ingestion of food, the small intestine is the first organ exposed to the digested nutrients. The intestinal epithelium, the initial site of intestinal absorption, is highly renewable which allows it to respond and adapt to changes in nutrition. Studies in rats have shown fasting promotes mucosal atrophy as early as 48-72 hours following the start of fasting, while 24 hour refeeding then induces intestinal growth and reverses the effects of fasting.\textsuperscript{28, 29} The highly renewable capacity of the intestinal epithelium is driven by the intestinal epithelial stem cells (IESC), located at the base of the intestinal crypt, while the villus serves as the functional unit predominately consisting of absorptive enterocytes (Fig 1). IESC divide to self-renew and give rise to daughter progenitor cells, which undergo rapid multiple cell divisions before differentiating
into one of four lineages: absorptive enterocytes, secretory Paneth, goblet, or enteroendocrine cells (EEC). Previous studies in *Drosophila* have shown that IESC respond to changes in fasting and refeeding by altering cell number and proliferation. Effects of ADF dietary interventions on the intestinal epithelium and IESC have yet to be defined.

The current study aims to investigate the impact of ADF on the intestinal epithelium. This investigation will specifically analyze phenotypic changes in mice, differences in intestinal crypt morphology, and changes in proliferation and mRNA levels due to long term ADF exposure. Expression of several genes, of which are implicated in intestinal proliferation, will be used to explore potential mechanisms by which changes may be occurring as a result of the ADF diet regimen. All segments of the small intestine and colon will be analyzed in the study, with a focus on the jejunum, the primary site of intestinal nutrient absorption. Given previous research showing decreased cell proliferation in several tissues from ADF, we hypothesize that ADF will result in decreased proliferation in the epithelium leading to changes in gene expression associated with intestinal growth and metabolism.
MATERIALS AND METHODS

Animals/Diet

Adult CD-1 mice (8 weeks old) were randomly divided into 2 groups: control and ADF. The control group received standard chow *ad libitum*, and the ADF group received the same diet given every other day. For a 24-hour period the ADF mice received food *ad libitum* followed by the removal of food during the next 24 hours. Food was removed and added at the same time every day. Water was given *ad libitum* for both groups. Measurements of body weight were performed weekly and food intake was recorded by subtracting the weight of the food in the cage from the amount of food added in the 24 hours prior to that point. After 20 weeks on ADF regimen, mice were euthanized. Prior to tissue collection, all ADF mice were euthanized following a 24-hour fast period. Controls were also fasted for the same 24-hour fast period to account for the short term impact of fasting.

Tissue Collection

All animals were euthanized following a 24-hour fast allowing for comparison between groups. Gonadal fat was dissected and weighed. Small intestine and colon was removed, cleaned and flushed prior to measuring weight and length. Small intestine was divided into three segments, the proximal 10 cm was considered duodenum, the distal 10 cm was considered ileum and the remaining segment was labeled the jejunum. Colon was divided in half and labeled proximal and distal colon.

Crypt Cell Count

H&E (haemotoxylin and eosin) staining of the cross sections of small intestine was performed for all animals. Number of cells in jejunal crypts was counted by H&E staining at 100x magnification.
For each animal, 30 open crypts were chosen and the number of nuclei within the crypt was counted.

**RNA Extraction and reverse transcription**

Total RNA was extracted from frozen tissue of the small intestine and colon tissue using the RNeasy mini kit following homogenization in Lysing Matrix D tubes with RLT Buffer and 1% β-mercaptoethanol. Purity and integrity of the extracted RNA was confirmed by electrophoresis in agarose gels containing ethidium bromide. Between 0.5 and 1.0 µg of pure, non-degraded RNA were used to synthesize cDNA with the High Capacity cDNA Reverse Transcription kit, including RNase inhibitor.

**qRT-PCR**

Platinum Quantitative PCR 2X Supermix –UDG and Taqman primer/probe sets were used to perform the quantitative real time polymerase chain reaction (qRT-PCR). A standard curve was made by pooling equal volumes of cDNA from all samples and run in each reaction. Samples were run in duplicates with less than 0.5 Ct of variation to determine gene expression using the StepOne Plus Real-Time PCR System. Expression values were all normalized to the invariant control, *Tbp*. Primer/probe sets for the following genes were used: TATA binding protein (*Tbp*), insulin-like growth factor 1 (*Igf1*), insulin receptor (*Ir*), insulin-like growth factor receptor (*Igf1r*), cyclin D1 (*Ccnd1*), and proglucagon receptor (*Gcg*)).

**RT-PCR for insulin receptor isoforms**

RT-PCR for insulin receptor (IR) isoforms was performed using primers that span exon 11 of insulin receptor mRNA so both IR isoform A (IR-A) and IR isoform B (IR-B) could be amplified in the same reaction. Products were run on a 2.5% agarose gel and 2 distinct bands (214bp – IR-A; 250bp – IR-B) were revealed. IR-B:IR-A ratios were calculated from values obtained by
measuring densitometry using ImageJ software, available through the National Institutes of Health (http://rsbweb.nih.gov/ij/).

**Statistical analysis**

Data expressed as mean ± SEM. A total of 8-9 animals were included in entire study. For all molecular experiments a subset of the animals (n ≥ 3) were included. Differences between diets were determined by Student’s t-test. P<0.05 was considered statistically significant.
RESULTS

**ADF decreases gonadal fat mass and plasma triglycerides despite no differences in body weight compared to controls**

After 20 weeks on the diet, body composition of the animals was assessed. No differences in body weight between control and ADF mice were observed (Fig. 2A). However, ADF mice had significantly decreased gonadal fat mass compared to controls (1.0 ± 0.2g vs. 0.4 ± 0.2g; Fig. 2B-C). Plasma triglyceride levels were also significantly decreased in ADF mice compared to controls (Fig. 2D).

**Significant decrease in jejunal crypt depth and crypt cell number of ADF group compared to controls but no change in villus height**

No difference in jejunal villus height was observed between groups (Fig. 3A). Jejunal crypt depth was measured and shown to be significantly decreased in ADF mice compared to controls (Fig. 3B & 3D). This decrease in crypt depth was associated with significant decreases in number of cells per crypt in ADF mice versus controls (Fig. 3C & 3D).

**Significant decrease in the number of EdU positive cells in ADF group compared to controls**

To evaluate changes in proliferation, mice were subjected to a 90-minute pulse of EdU prior to euthanasia to mark cells in S-phase. Quantification revealed a significant decrease in the number of EdU positive cells in ADF mice versus controls (8.5 ± 0.3 vs. 6.8 ± 0.5; Fig. 3E & 3F).

**No significant change, but trend for decrease in Igf1, Igf1r, Ir, Gcg, and Ccnd1 mRNA in the jejunum of ADF group versus fasted controls; Significant increase in IR-B:IR-A ratio in jejunum of ADF group versus fasted controls**

Gene expression analysis of potential mediators of intestinal proliferation was performed to elucidate the underlying mechanism in ADF mice. Because of its roles in intestinal proliferation and nutrition, we wanted to assess if intestines from ADF mice displayed changes in the key genes involved in the IGF1/insulin pathway. Using qRT-PCR, mRNA of the following genes was measured in the small intestine and colon: *Igf1, Ir, Igf1r, Ccnd1 and Gcg*. All segments of the
small intestine were analyzed, however, we concentrated on the jejunum, the primary site of intestinal nutrient absorption. We observed no significant changes, but a trend for decrease in mRNA levels in jejunums between groups (Fig. 4A). The IR has two distinct isoforms, IR-B and IR-A, which play counter-regulatory roles in proliferation. The mRNA levels of both isoforms were measured and a significant increase in the IR-B:IR-A mRNA ratio was found in the jejunum of ADF group compared to fasted controls (Fig. 4B & 4C).

**Significant decrease in Igf1, Igf1r, Ir, and Ccnd1 mRNA and increase in IR-B:IR-A ratio in the colon of ADF group versus fasted controls**

When we looked at Igf1, Ir, Igf1r, Ccnd1 mRNA levels in the colon, we found drastic decreases in Igf1, Ir, Igf1r, and Ccnd1 mRNA of ADF group compared to controls (Fig. 5A-D). A significant increase in the IR-B:IR-A mRNA ratio was also observed in the colon of ADF group compared to controls (Fig. 5E & 5F).
DISCUSSION

ADF has become popular within the last decade, as it has proved to have many similar physiological benefits to CR yet with possibly more feasibility as a diet regimen. In addition to those beneficial physiological effects, decreases in cell proliferation in many tissues have been observed after treatment with ADF. However, the impact of ADF on the intestinal epithelium, specifically in regards to proliferation, has been unexplored. As a result, the question addressed in the current study was how ADF impacts the intestinal epithelium. We have shown in the intestine that ADF results in decreased jejunal crypt depth and crypt cell number, as well as an increased shift towards expression of the IR-B isoform of the insulin receptor, which is anti-proliferative and promotes differentiated function.\(^3\) Results from the colon showed decreased expression of important genes in the IGF1/insulin pathway, which promotes intestinal epithelial growth. A trend for decrease in those genes was also observed in the small intestine. With the summary of these results we propose that ADF reduces proliferation and promotes differentiated function in the intestine, which allows for increased efficiency in harvesting nutrients per unit length of the small intestine and protection against tumorigenesis in the colon.

We observed a significant decrease in crypt depth in jejunum of ADF mice and this was associated with similar decreases in crypt cell number, indicating more shallow crypts were due to a decrease in cell number. Changes in crypt depth but not in villus height suggests intestines of ADF mice may adapt to the diet by decreasing the proliferative compartment without sacrificing functional capacity.

Another impact of the ADF diet regimen on the small intestine was a decrease in the number of proliferating cells per crypt. This was illustrated by the decreased number of cells labeled by EdU, which marks all cells in S-phase during the 90 minute pulse in which mice were
subjected to it. This decreased number of proliferating cells may be an adaptation to spare the functional cells within the crypt, so that absorption of nutrients can be more efficient when the fasting period is over and *ad libitum* feeding beings. This decrease in intestinal epithelial cell proliferation is supported by previous research which has shown ADF diet regimens, consisting of a 24 hour fast and 24 re-feeding regimen over a 4 week time period, promote significant decreases in proliferation of epithelial, splenic-T, and mammary epithelial cells.\textsuperscript{26}

In the current study, although not significant, we observed a trend for a decrease in key genes within the IGF1/insulin pathway in the jejunum. The analyzed pathway intermediates include *Igf1, Igf1r, Ir, Gcg*, and *Ccnd1*. We analyzed the expression of these genes implicated in the IGF1/insulin pathway to find possible mechanisms by which ADF resulted in decreased intestinal epithelial cell proliferation. The IGF1/insulin signaling pathway has been shown to play a critical role in regulating the growth of the intestinal epithelium, and is affected by changes in nutrient status within the intestinal lumen.\textsuperscript{31,32} The insulin-like growth factor 1 receptor (*Igf1r*) is highly homologous to the insulin receptor (*Ir*), allowing both IGF1 and insulin to be able to signal through the pathway to proliferation.\textsuperscript{30} It has also been shown that proglucagon (*Gcg*) acts through IGF1 to promote proliferation,\textsuperscript{33} and that IGF1 upregulates cyclin D1 (*Ccnd1*), which forms a complex necessary for regulating the progression of the G\textsubscript{1} phase of the cell cycle.\textsuperscript{34} Further research will be done with a larger sample size to observe if there is a significant downregulation of these intermediates within the IGF1/insulin pathway in the jejunum of ADF mice.

The IR exists in two structurally different isoforms, IR-A and IR-B. The differing factor is the presence (IR-B) or lack (IR-A) of exon 11, located at the C-terminus ligand-binding α-subunit of the IR. Recent research suggests IR-A is predominantly expressed in cycling IESC and rapidly dividing progenitors, while IR-B is highly expressed in post-mitotic differentiated
lineages. As a result, the IR isoforms are thought to modulate proliferation by either promoting IR-A or inhibiting IR-B expression. Because of the importance of the IR isoforms in regulating proliferation, their mRNA levels were measured. While we see no differences in expression in total IR, ADF shifts the IR isoform ratio to favor IR-B expression which is anti-proliferative and promotes differentiation. This shift towards IR-B expression suggests an anti-proliferative effect of ADF on the small intestine, as well as an increase in differentiated function. The combination of inhibited proliferation and promoted differentiated function may be an adaptation of the small intestine to the ADF regimen, allowing it to maximize its ability to harvest nutrients per unit length, while decreasing expenditure of energy on growth.

Analysis of gene expression in the colon was surprising in that it showed several significant changes of which were not observed in the small intestine. In the colon, expression of Igf1, Igf1r, and Ir, were significantly downregulated compared to controls. This downregulation of genes in the IGF1/insulin pathway in the colon supports the conclusion that ADF has anti-proliferative effects on the colon, in addition to the jejunum. This is also demonstrated by the significant increase in the IR-B:IR-A ratio in the colon. IR-A has been both linked to tumorigenesis and shown to be increased in intestinal tumors and colorectal cancer cell lines. Therefore, a reduction in IR-A may be protective against tumorigenesis in the colon, which is augmented by the increase in the anti-proliferative effects of IR-B. Decreased expression of Ccnd1 further supports the anti-proliferative effects of ADF. Taken together, our data shows that ADF has a larger effect on the colon and may be protective against tumorigenesis. This may be another similar link between ADF and CR, given that several studies have shown CR reduces risk of colon cancers.

The results of this study support the preliminary data showing ADF does not result in significant changes in body weight compared to controls. However, significant decreases in
gonadal fat and TAG levels in the ADF group were shown. These differences may suggest changes in energy utilization and expenditure between ADF and control mice. Another possible reason for these differences may be related to changes in fat storage due to the microbiome. Previous research, comparing germ-free and conventionally raised mice, has shown gut microbiota act as an environmental factor that regulates the storage of fat. Further research will be done to analyze the impact of ADF on the microbiome.

There were some limitations to the study. The sample size for molecular analysis was small (n=3), and may account for why certain changes in gene expression in the small intestine were not significant. However, the drastic significant decreases in the colon with this limited number signifies the results seen in the colon are consistently true. Another limitation was that upon completion of the 20 week diet, mice were euthanized after a 24 hour fast. Changes may have been more apparent as different if ADF mice had been euthanized after a day of feeding. The length of the diet is another possible factor that could have affected the results. Because the intestine is so highly adaptive, a shorter length of the diet may have shown more significant differences between the treatment and control groups. Lastly, there were no changes in body weight between the treatment and control mice, which may have produced different results than if body weight had decreased after treatment. In one ADF study, circulating levels of IGF1 were reported to increase after 20 weeks of treatment, yet another study reported a significant decrease after 24 weeks of treatment. A major difference between these two studies was that animals did not lose weight in the 20 week study, yet they did in the 24 week study.

In conclusion, the results suggest ADF has anti-proliferative effects and promotes differentiated function, which may allow for increased absorptive efficiency per unit length of the
small intestine and protection against tumorigenesis, particularly in the colon (Fig. 8). Future efforts will look at changes in the microbiome in response to an ADF diet regimen.
FIGURE LEGENDS

**Figure 1:** Intestinal epithelium is organized into crypts containing stem, Paneth, and progenitor cells and villi containing functional differentiated lineages

**Figure 2:** ADF decreases gonadal fat mass and plasma triglycerides despite no differences in body weight compared to controls. (A) Measurements of body weight over 20 weeks on the control or ADF diet. (B) Image of gonadal fat of control and ADF mice. (C) Gonadal fat mass of control and ADF groups. (D) Plasma triglyceride levels of control and ADF groups. n≥3 per group; *p<0.05 vs. control; unpaired t-test

**Figure 3:** Significant decrease in jejunal crypt depth, crypt cell number and number of EdU positive cells in ADF group compared to controls. (A) Villus height for control and ADF groups. (B) Crypt depth for control and ADF groups. (C) Number of cells in jejunal crypts. (D) Image of H&E stained crypts at 10x and 40x (inset). E-F: Immunofluorescence image (E) and quantification (F) of EdU (red) and DAPI (blue) reveal significant decrease in number of EdU positive cells in ADF mice compared to controls. Images taken at 40x. n≥4 per group; *p<0.05 vs. control; unpaired t-test

**Figure 4:** No significant changes, but a trend for decrease jejunal mRNAs involved in the IGF1/insulin pathway, however, significant shift favoring IR-B:IR-A ratio. (A) qRT-PCR measured mRNA levels of *Igf1, Igf1r, Ir, Gcg* and *Ccnd1* for ADF and control groups. (B) Representative gel from RT-PCR assessed the ratio of IR-B (top band) and IR-A (bottom band) mRNAs using primers spanning exon 11 of the *Ir*. (C) Quantification of the band intensity reveal significant up-regulation in IR-B:IR-A in ADF mice. n≥3 per group; *p<0.05 vs. control; unpaired t-test
Figure 5: Significant decrease in Igf1, Igf1r, Ir, and Ccnd1 and an increase in IR-B mRNA in the colon of ADF group versus fasted controls. A-D: qRT-PCR measured mRNA levels of Igf1 (A) Igf1r (B) Ir (C) and Ccnd1 (D) in colons of ADF versus control mice. E-F: Representative gel from RT-PCR displaying IR isoform expression in proximal and distal colon (E) and quantification of band intensity (F). n=4 per group; *p<0.05 vs. control; unpaired t-test

Figure 6: Proposed model of ADF effects on small intestine, colon and adipose tissue. ADF acts on the intestine to maximize nutrient harvest in the small intestine and to limit intestinal epithelial growth in both small intestine and colon. ADF may also modulate adiposity through microbiome changes.
REFERENCES

1. Weiss EP, Racette SB, Villareal DT, et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr 2006;84:1033-42


FIGURES

Figure 1

Crosnier et al., 2006
Figure 4
Figure 5
Figure 6

Alternate day fasting (ADF)

Small intestine
- IR-B
- IR-A
Andres et al., J Cell Sci, 2013

- Increased differentiation
- Increased sucrose isomaltase
- Increased lipid/cholesterol handling

- Increased efficiency to harvesting nutrients
- Decreased crypt depth
- Decreased growth
- Increased EdU+ cells

Maximizes ability to harvest nutrients while decreasing growth

Colon
- IR-B
- IR-A
Andres et al., J Cell Sci, 2013

- Limits proliferation

- IR-A linked to tumorigenesis
- IR-A increased in intestinal tumors and colorectal cancer cell lines

Adipose tissue
- IR-B
- IR-A

- Decreased luminal nutrient due to increased harvest in SI

Changes in microbiota composition that protects against adiposity
- Shift favoring IR-B expression may be protective against tumorigenesis