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# Intra-abdominal Adipose Tissue as a Major Source of IL-6 in Experimental Colitis

W. Conan Mustain, MD

# Adipose tissue – What is "fat"?

- Heterogenous tissue composed of mature adipocytes, preadipocytes, endothelial cells, macrophages, leukocytes, and fibroblasts

- In addition to energy storage, adipose tissue is now known to have important endocrine role in metabolism, immunity, and inflammation.

- Over 50 known adipokines including inflammatory cytokines (TNF-α, IL-6, MCP-1), growth factors (IGF-1, VEGF), and adipocyte-specific "adipormones"

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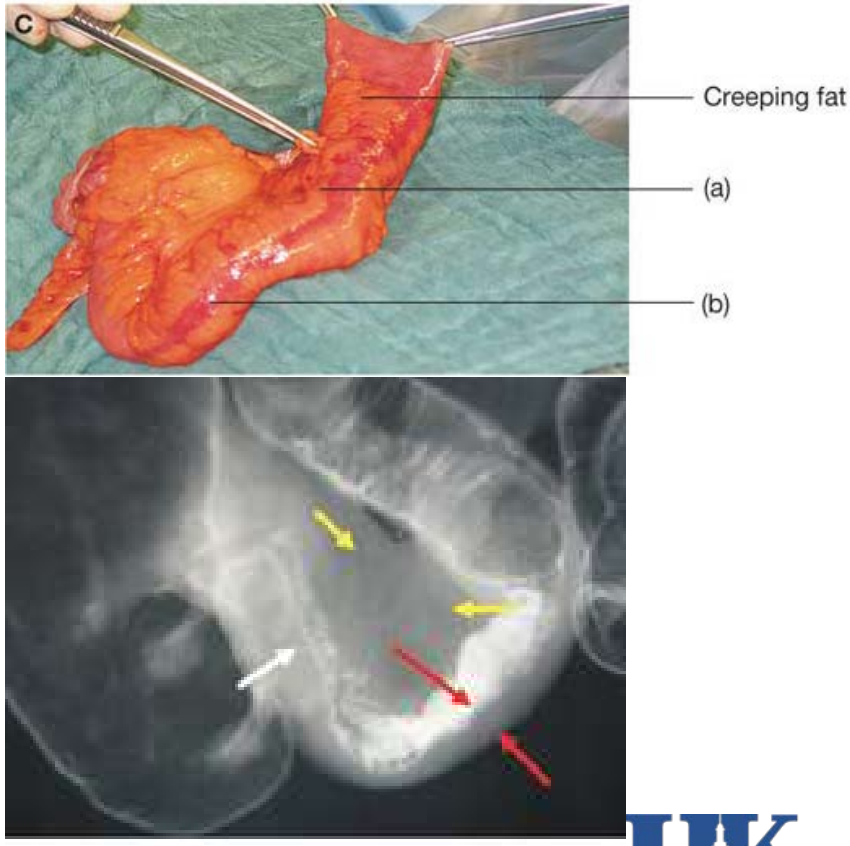
# Adipose tissue and Inflammation

- Obesity is now recognized as a chronic inflammatory condition
  - Macrophage infiltration of adipose tissue
  - Increased circulating CRP, IL-6, and adipokines
  - Increased fecal calprotectin levels
- Obesity-associated diseases are increasingly attributed to inflammation
  - Atherosclerosis
  - Prevalence of colorectal cancer
  - Severity of acute pancreatitis
- Visceral fat correlates with disease states more strongly than total body fat

# Adipose tissue and IBD

- Hypertrophic mesenteric adipose tissue and fat-wrapping are hallmarks of Crohn's disease
  - higher levels of TNF-a, IL-6, MCP-1, leptin, resistin, and adiponectin than healthy subjects
- Obesity associated with higher year-by-year disease activity in Crohn's

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# Obesity and IBD

## Prevalence and Epidemiology of Overweight and Obesity in Children with Inflammatory Bowel Disease

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- Prevalence of obese/overweight in pediatric IBD population is 23.6% (20% in CD, 30.1% in UC)

?

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# Questions

- Does induction of colitis result in increased expression of inflammatory cytokines from adipose tissue in an animal model of IBD?

- Does intra-abdominal fat respond differently than subcutaneous fat?

- Do adipose-derived cytokines contribute to severity of colitis?

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# Methods

- Model
  - 2% dextran sulfate sodium in drinking water for 5 days
  - C57BL
- Mice sacrificed at Day 0, 3, 7, 14, and 21
- Outcomes
  - Representative animal for histology
  - Plasma cytokines by multiplex
  - Tissue mRNA levels by qRT-PCR

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# Results – Severity peaks at Day 14

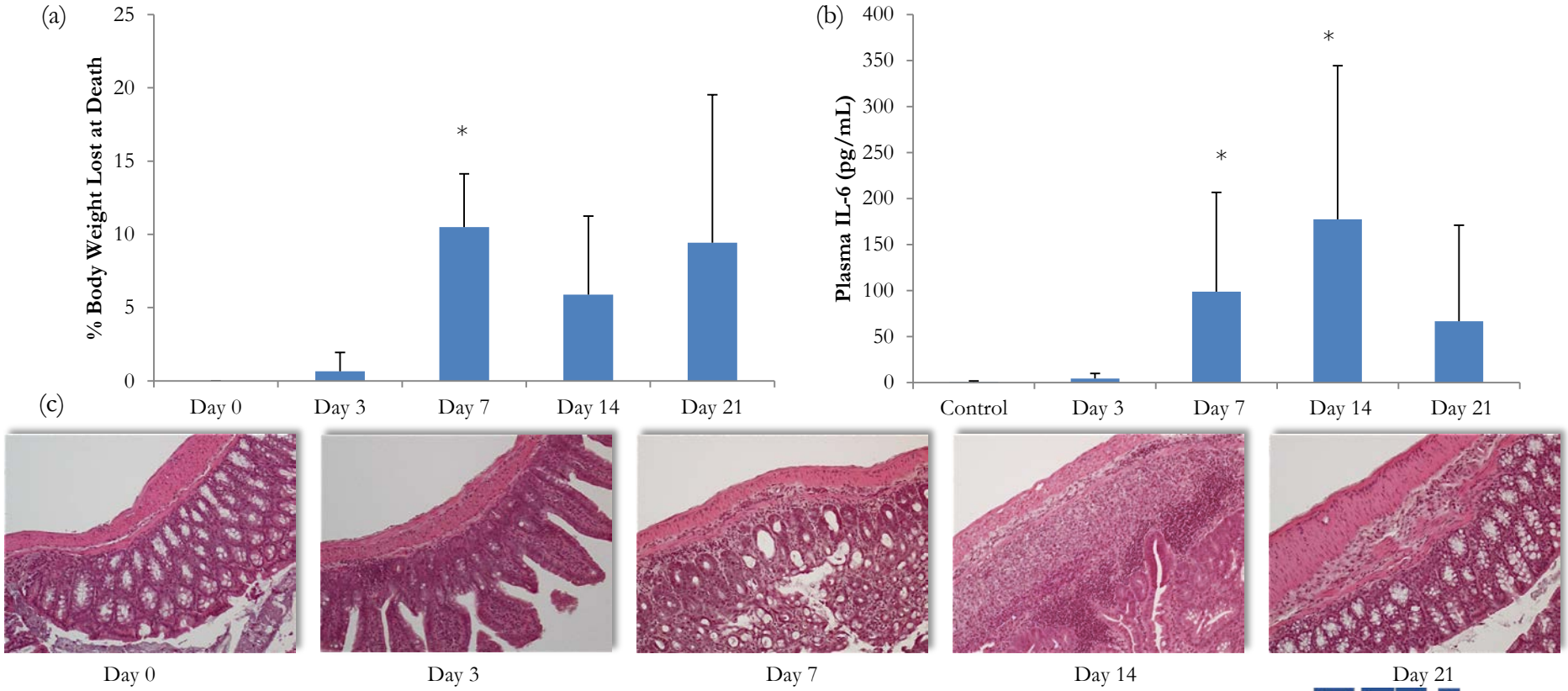


**Fig 1:** After treatment with DSS, mice experienced significant decreases in body weight (a). Increases in Plasma IL-6 levels (b) and histologic evidence of colonic inflammation (c) were evident by Day 7 and peaked at Day 14.

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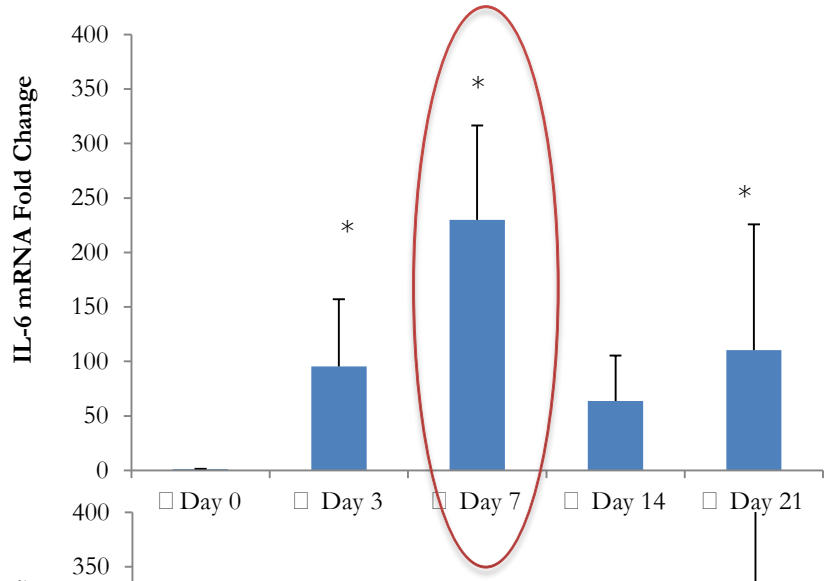
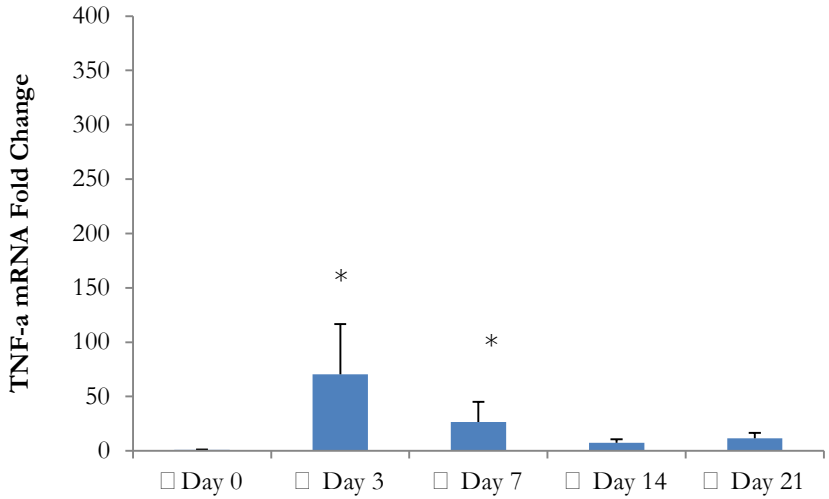
# Cytokine Expression in Colon

(a)




(b)




(c)

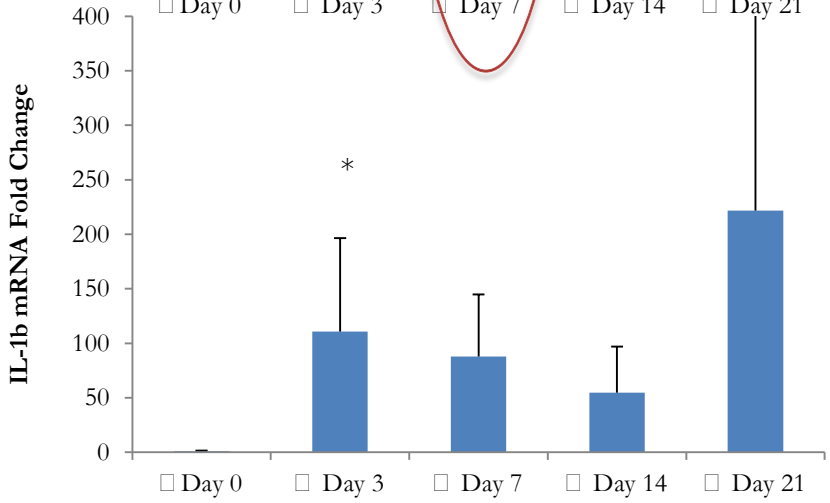



**Fig 2.** Colon mRNA levels of IL-6, TNF-α, and IL-1β were significantly increased by DSS treatment as early as Day 3. IL-6 levels were increased by 230 fold at Day 7 (a) making this the most prominent cytokine induced in colonic tissue.

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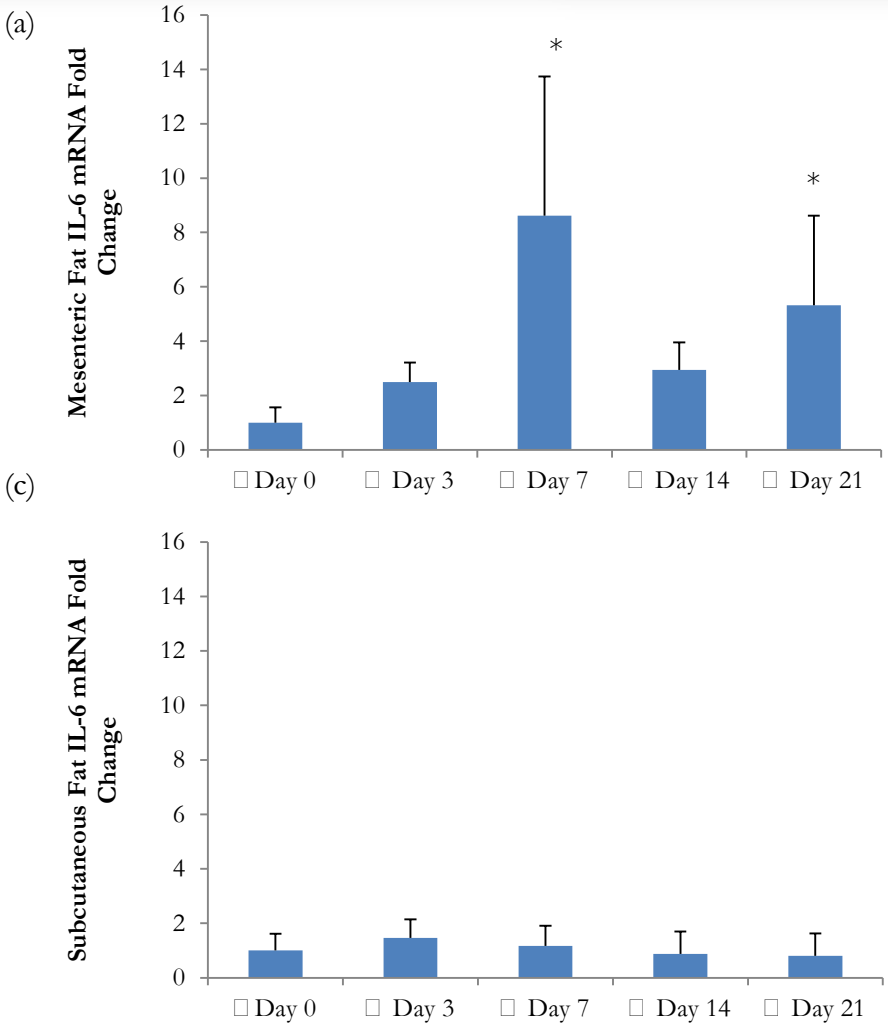
# IL-6 is induced in intra-abdominal adipose tissue



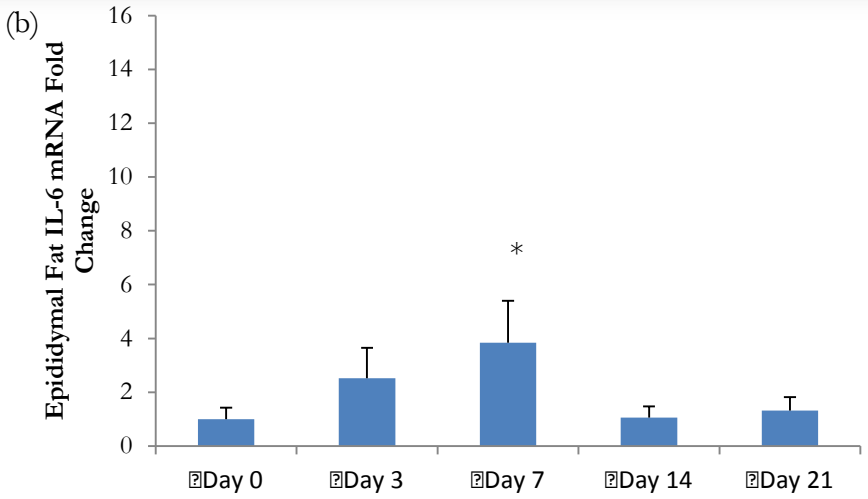




**Fig 4. (a,b)** IL-6 mRNA levels in Mesenteric (8.6 fold) and Epididymal (3.8 fold) Fat at Day 7 were significantly increased from baseline **(c)** No significant increase in Subcutaneous Fat IL-6 expression occurred with treatment. **P<0.05*

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# IL-6 levels at Day 7

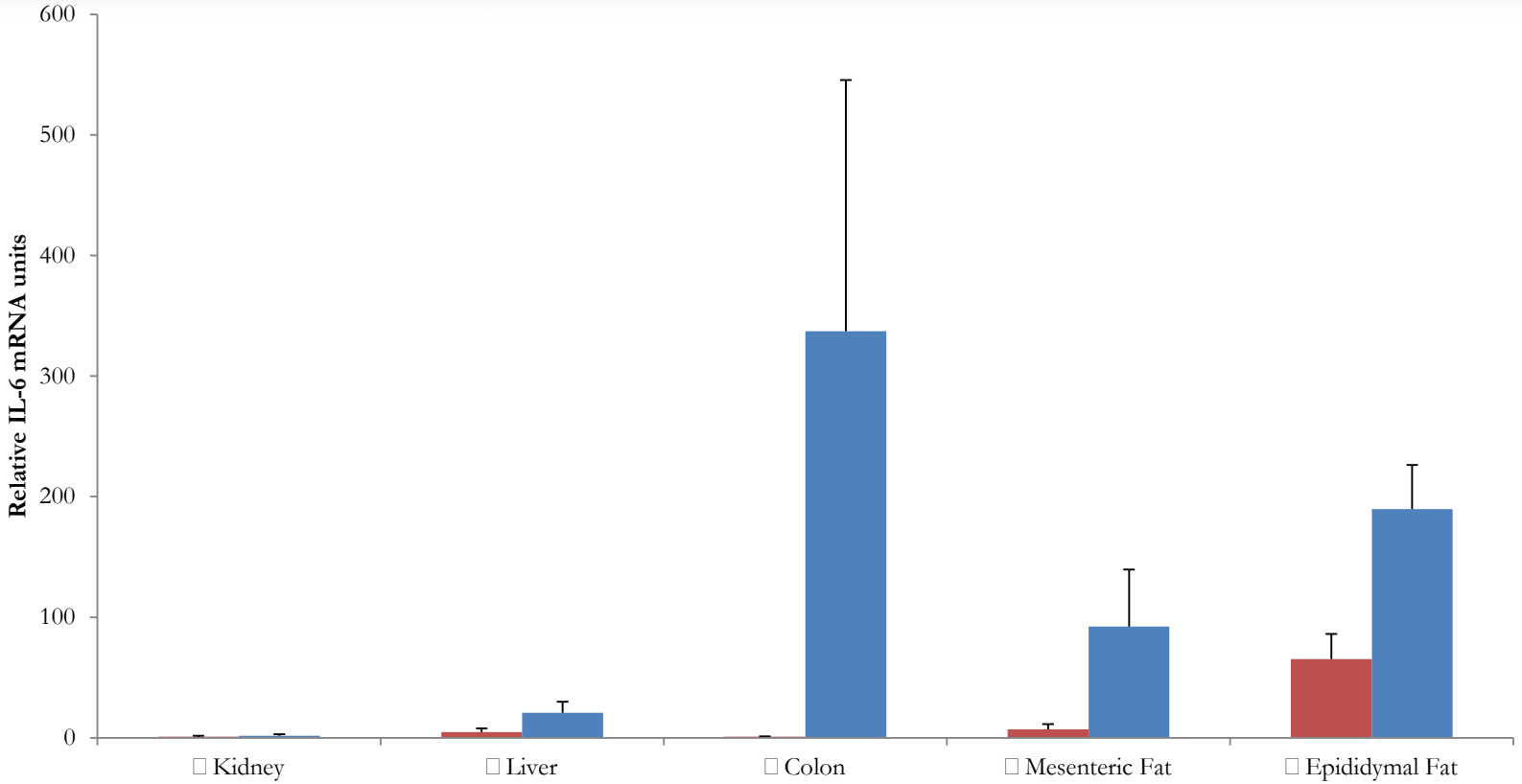


**Fig 5.** Day 7 mRNA levels of IL-6 are significantly increased above control in colon, mesenteric fat, and epididymal fat. Adipose tissue levels at Day 7 are significantly higher than kidney or liver.

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# Preliminary Conclusions

- During acute experimental colitis, adipose tissue is a major source of IL-6 production.
- IL-6 is significantly induced from intra-abdominal, but not subcutaneous adipose tissue
- Peak in adipose-derived mRNA precedes peak plasma levels, suggesting a contribution by adipose tissue to circulating levels
- Epididymal fat pad changes suggest tissue-specific response rather than mere local lymphoid reaction to tissue injury

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# Further Questions/Plans

- Does adipose-derived IL-6 contribute to the severity of colitis?
  - Mice fed a high-fat diet have been shown to have more severe response to DSS
  - Preliminary data suggesting improved survival in SIRS with removal of epididymal fat
- Compare severity of colitis between caloric-restricted and high-fat diet mice, with and without removal of epididymal fat pad.

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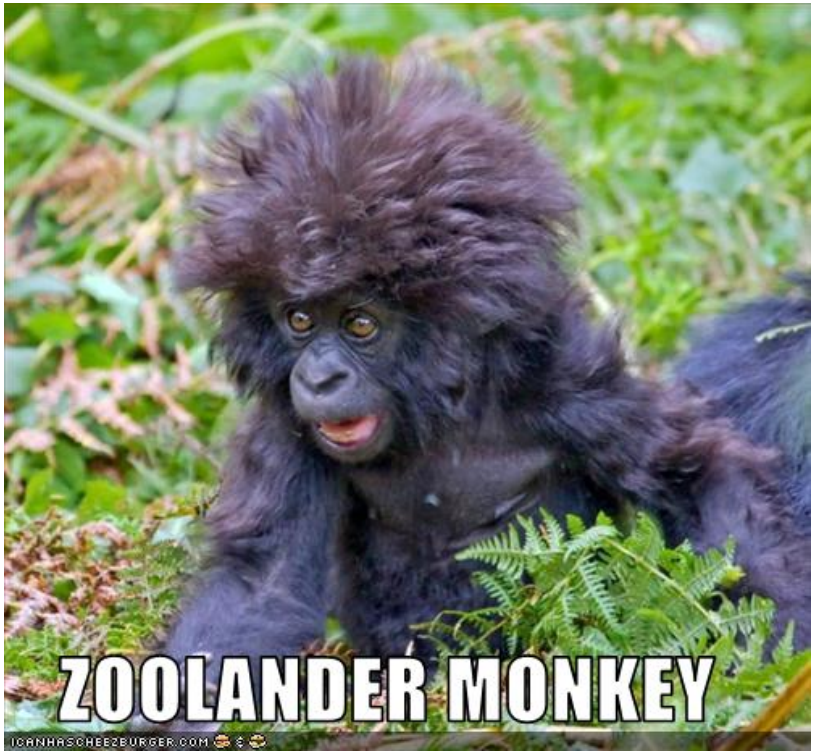
# The Use of High Resolution Colonoscopy for Development of a Novel Orthotopic Murine Model of Colorectal Cancer

# Animal models

- Needed to study mechanisms of pathogenesis but also to assess potential therapies
- Cannot recapitulate all aspects of human disease
- Advantages and limitations depending on outcome of interest

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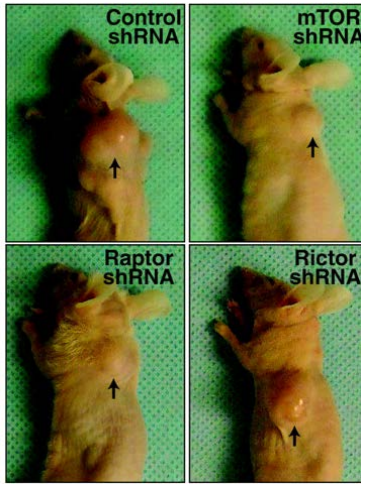
# Murine Models of Colorectal Cancer

- Sporadic Models
  - Genetically-engineered (*Apc* mutant, MutS/MutL, *Muc2-/-)*
  - Chemically-induced (AOM/DSS)
- Transplant Models
  - Heterotopic Xenografts
  - Metastasis Assays (IV or intra-splenic)
  - Surgical Orthotopic Implantation

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# Purpose

- Establish an orthotopic model of colorectal cancer via intramural cell injection using high-resolution colonoscopy

- Theoretical advantages
  - Orthotopic location
  - Minimally-invasive
  - Serial evaluation

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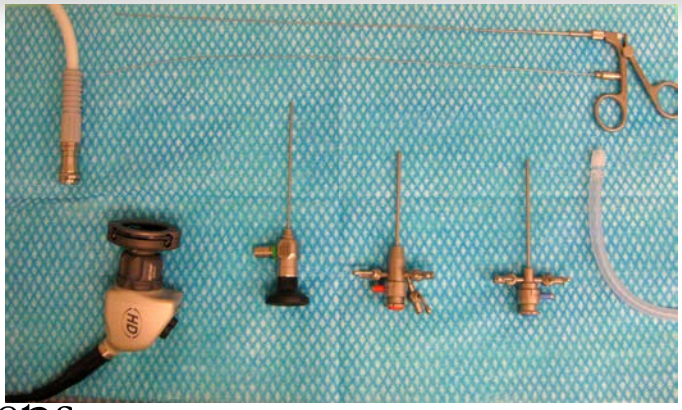
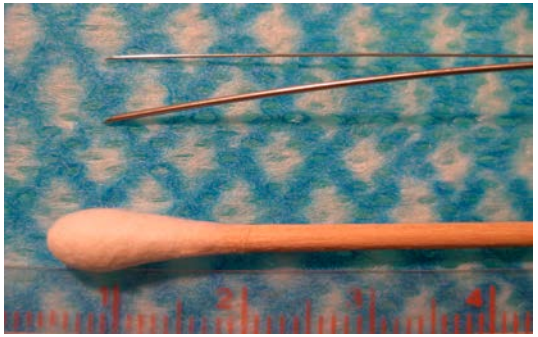
# Materials



- Equipment
  - Mini-endoscope
    - Karl-Storz Coloview ®
    - 1.9mm scope, sheath, biopsy forceps
  - Standard laparoscopic set-up
    - Donation from Stryker ®
  - Custom injection needle and Microliter syringe
    - Hamilton® Company
    - 6” 30G needle



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# Methods

- Cell suspension prepared in phosphate-buffered saline
- Mice anesthetized with ketamine-xylazine
- Scope inserted to mid-descending colon
- 25 mL cell-suspension injected into submucosa
- Weekly surveillance with colonoscopy

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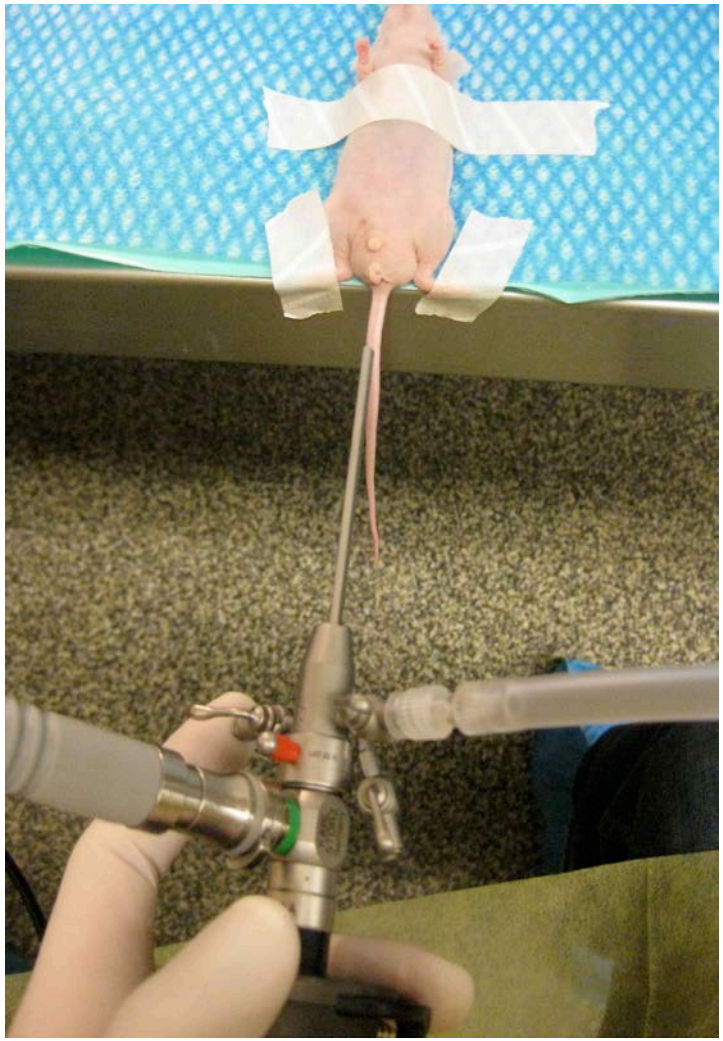
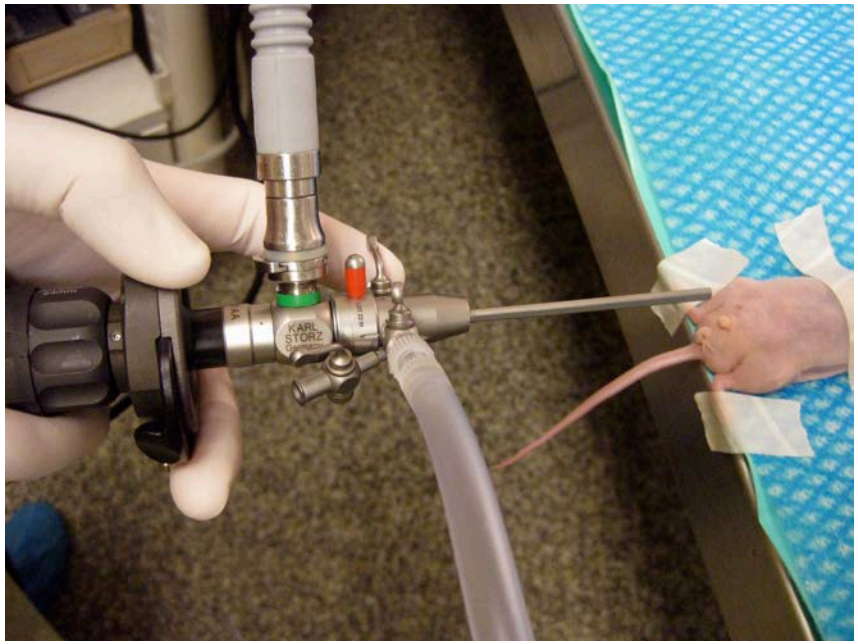
# Procedure







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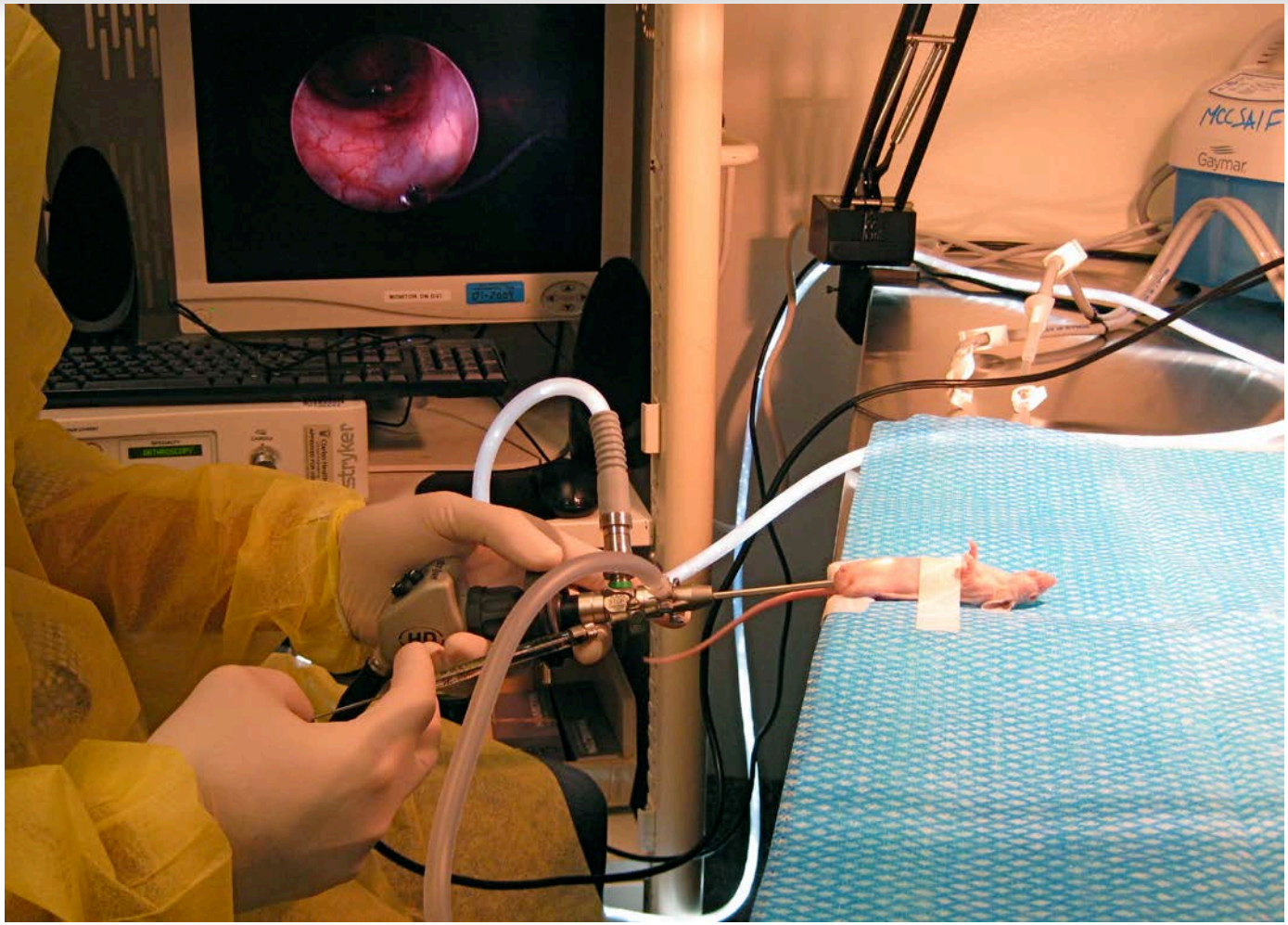
# Procedure

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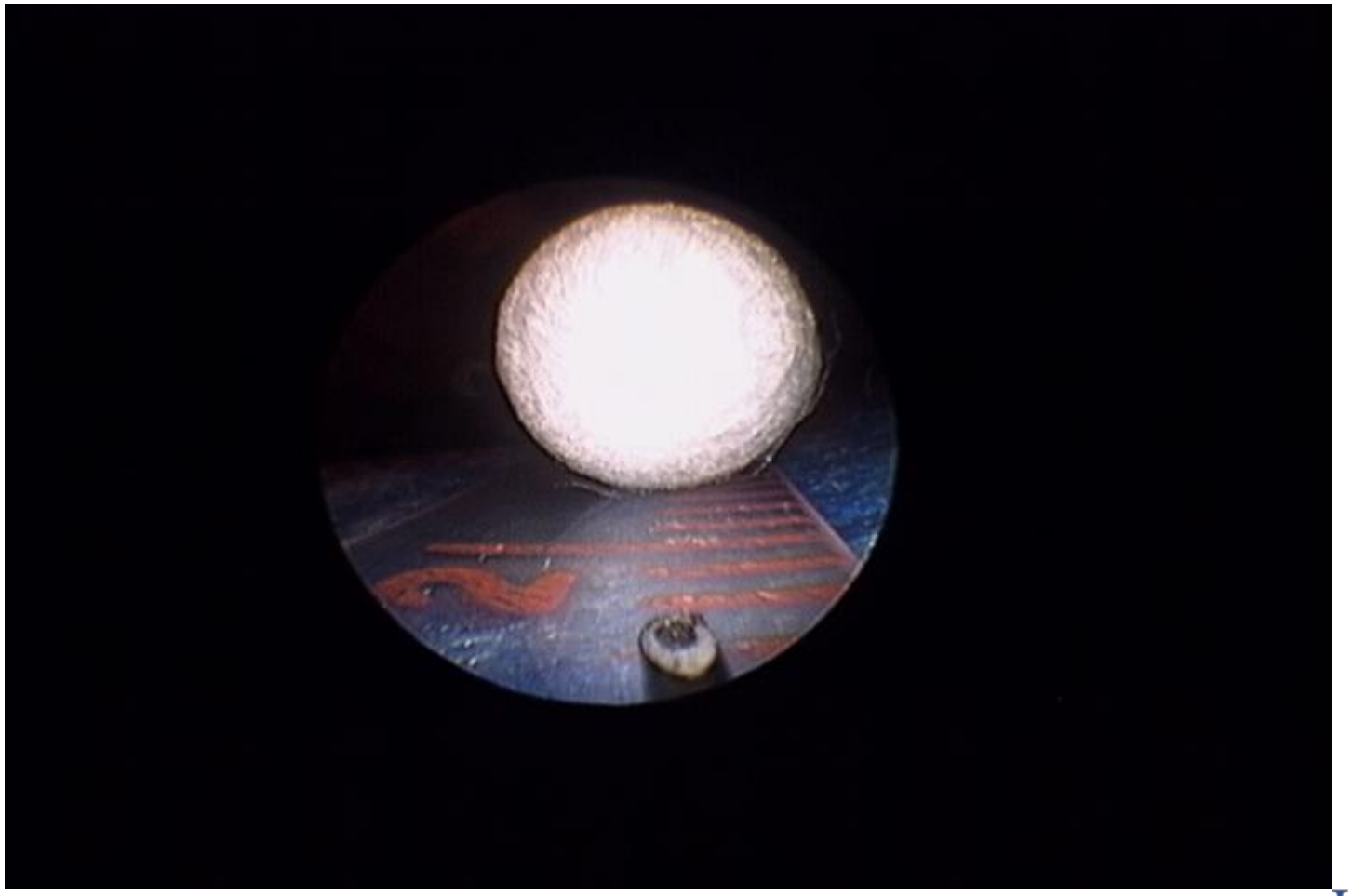
# Methods



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# Video

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# Results

- Procedure tolerated well
  - 1 death due to perforation
- Tumors first visible at 14 days
- Histology confirmed tumor establishment in submucosa
- Tumor establishment rate of 83% (25/30 injections)

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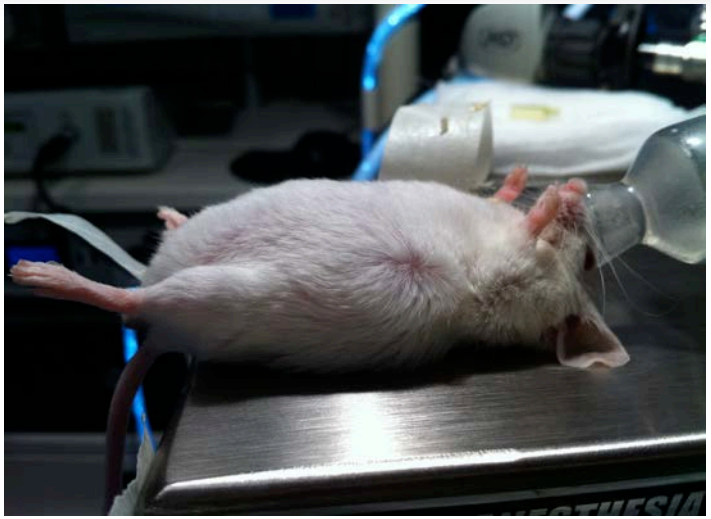
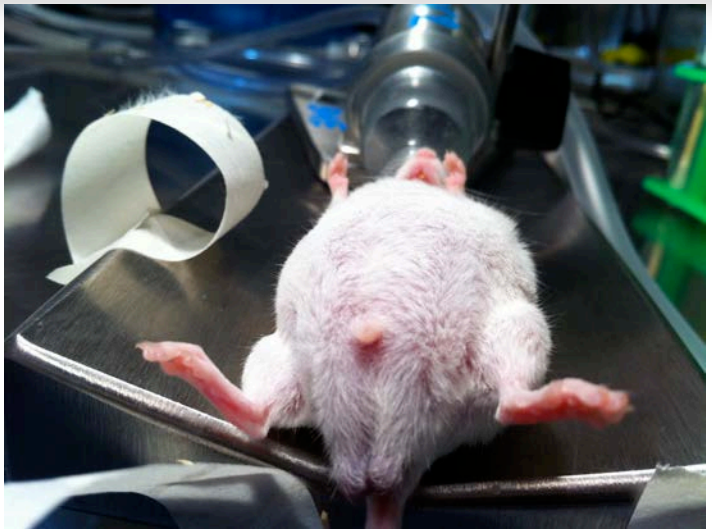
# Complication





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# Conclusions

- Intramural injection in descending colon is possible
- Tumor growth can be reliably monitored on serial endoscopy
- Successful tumor establishment with multiple cell lines in immunocompetent and immunodeficient mice

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# Conclusions

- Disadvantages/Shortcomings
  - Technically challenging
  - Growth duration limited by obstruction
  - Inconsistency precludes comparative studies
- Applications
  - Cancer cell interactions with microenvironment
  - Tissue-specific gene knockout with Cre-recombinase

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