Westernized Diet-induced Insulin Resistance in Mice is Associated with Focal Duodenal Hyperplasia

S. Ghosh[1], F. Rubino[2], P. Wismann[3], K. Rigbolt[3], H. Rajagopalan[4], D. Maggs [4]

[1]San Diego, CA; [2]King's College London, United Kingdom; [3]Gubra ApS, Hørsholm, Denmark; [4]Fractyl Laboratories Inc., Lexington, MA

Introduction

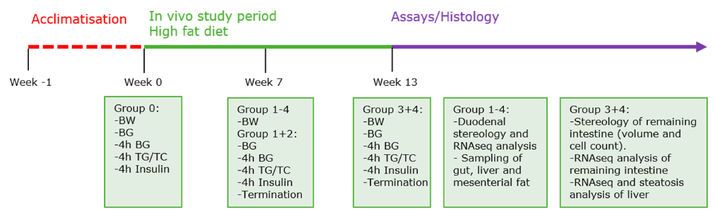
- A Westernized Diet (high fat and sugar) induces an insulin resistant state and is associated with obesity and type 2 diabetes (T2D).
- Duodenal exclusion surgery in obese T2D patients implicates the duodenum as a key GI region involved in propagating systemic insulin resistance.[1]

Objective

- To identify adaptive changes of pathophysiological relevance, detailed analyses of the GI tract was undertaken following exposure of mice to Westernized Diet.

Methods

- C57Bl/6J mice (male, 6 weeks of age) were exposed to either a lean (Altromin chow) or Westernized Diet (WD; 60% fat/ 20% sugar; D12492 SNIFF) for 7 and 13 weeks.
- Systematic metabolic measures were conducted over time including: plasma glucose, insulin, body weight, liver weight and fat content.
- Detailed stereological analysis was conducted along the length of the gastrointestinal tract (duodenum, jejunum, ileum, colon) with mucosal surface staining.
- Transcriptomic analysis of gut and liver tissue samples was also conducted.



BW = Body weight; BG = blood glucose; TG/ TC – triglycerides / total cholesterol; 10 Mice / cohort: Group 0 = Baseline; Group 1 = Lean diet 7 weeks; Group 3 = Lean diet 13 weeks; Group 2 = WD 7 weeks; Group 4 = WD 13 weeks

Results

Metabolic Measures

- Compared to mice fed a lean diet, mice fed a Westernized Diet:
  - Gained significantly more weight (Table 1).
  - Had significantly higher plasma glucose, insulin and total cholesterol levels (Table 1).
  - All differences were greater at 13 vs 7 weeks.

Table 1. Metabolic Changes in Mice Fed a Lean vs. Westernized Diet (WD)

Measure, mean (SEM)	Baseline	Lean 13 weeks	WD 13 weeks	P- value
Body weight*, g	20.9 (0.2)	27.6 (0.3)	44.9 (1.3)	<0.001
Fasting-blood glucose**, mmol/L	7.9 (0.5)	6.8 (0.4)	10.6 (0.5)	<0.001
Fastin -plasma insulin**, pg/ml	612. (69)	535 (50)	4,214 (818)	<0.001
Total cholesterol**, mmol/L	2.1 (0.1)	1.4 (0.05)	3.6 (0.2)	<0.001

*Two-way RM ANOVA, Bonferroni post hoc test; **One-way RM ANOVA, Bonferroni post hoc test. † ‡Student's t-test.

Liver and Duodenal Tissue Findings

- Compared to lean fed controls, mean duodenal weight and liver weight was significantly increased at 13 weeks in mice fed a Westernized Diet.
- Unlike other intestinal segments, focal hyperplasia was observed in the duodenum with increased weight and surface area (~40%) (Table 2).
- Duodenal mucosal volume was also significantly increased at 13 weeks (Table 2); however, submucosal + muscularis volume was unchanged (data not shown).

Table 2. Increased Duodenal Weight, Volume and Surface Area Observed in Mice Fed a Lean vs. Westernized Diet (WD)

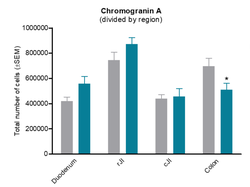
Measure, mean (SEM)	Lean 13 weeks	WD 13 weeks	P- value
Liver weight**, g	0.96 (0.02)	1.4 (0.1)	<0.001
Duodenal weight**, mg	152 (3.4)	172 (5.9)	<0.001
Duodenal mucosal volume†, mm³	120 (2.4)	130 (3.7)	<0.05
Duodenal surface area†, cm²	44.0 (1.6)	61.6 (3.4)	<0.001

Mean Total Whole Intestine Volume ±SEM estimated by stereology in mice fed a Lean vs WD after 13 weeks **p<0.01 vs. Lean 13 weeks, unpaired t-test

Chromogranin-A Staining

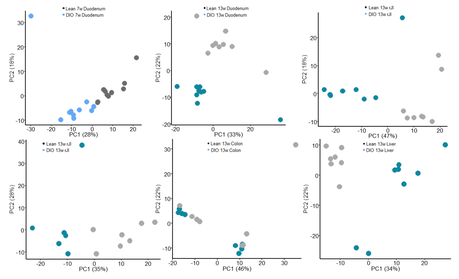
- The hyperplastic duodenum seen in Westernized-Diet fed mice was also characterized by increased chromogranin-A staining of enteroendocrine (EE) cells (Figure 1). Increased staining was also observed in the jejunum but not in ileum or colon.

Figure 1. Number of Chromogranin-A Cells per Region of the Gut



Whole intestine chromogranin A cell number ±SEM, sorted by intestinal region. Cell numbers were estimated by physical disector in mice following consumption of regular chow (lean) or high fat diet (DIO) for 13 weeks. *P<0.05 vs. Lean 13 weeks, unpaired t-test.

Figure 2. Principal Component Analysis (PCA)

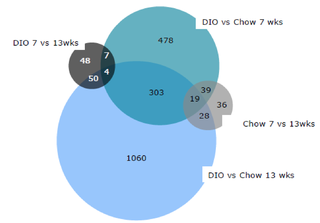


Points indicate the relationship between samples across their gene expression profile
Values in parenthesis indicates the amount of variability explained by axis.

Transcriptomic Data

- In a principal component analysis of the 500 most variable genes, distinct differences were observed between mice on lean versus Westernized Diet **(Figure 2).**
- In liver and small intestine tissues (duodenum, jejunum, ileum), a distinct division was observed between samples from mice fed a Westernized vs Lean Diet.
- Overall, mice fed a Westernized Diet had a greater number of differentially expressed genes (compared to Lean Diet) at 13 weeks vs 7 weeks **(Figure 3).**
- A pathway perturbation analysis was conducted based on the number of differentially expressed genes in a given pathway, divided by the total number of genes in the pathway. In this analysis, certain metabolic pathways were differentially altered across gut regions with duodenum signature differentiated from other gut regions and the liver.

Figure 3. Duodenal Transcriptomic Analysis: Shared Regulated Genes

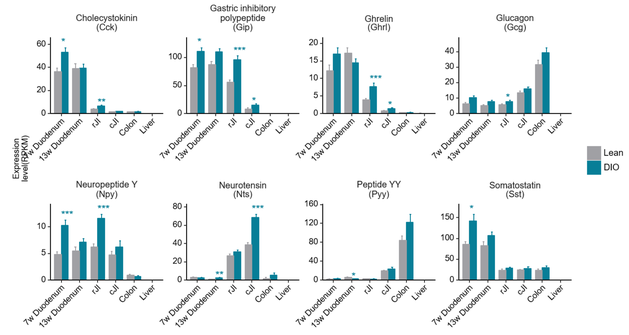


Venn diagram of differentially expressed genes in the duodenum only. Values represent the number of genes in a group and the size of circle represents the number of differentially expressed genes in each group. The overlap indicates the proportion of shared genes.

Gut Hormone Transcription Data

- Significant perturbations in expression of gut hormones were seen across segments of the gut (generally increased) in mice fed a Westernized Diet (Figure 4).
- Expression changes appeared to manifest regionality with some specific hormones being perturbed more so in duodenum and proximal gut.

Figure 4. Gut Hormone Transcriptomic Data



Average + SEM of expression levels of pathway subset genes. *: p< 0.05, **: p< 0.01, ***: p< 0.001 after correction for gene-wise multiple testing.

Conclusion

- Exposure to WD induces adaptive responses in duodenal mucosa, EE cell population, and the transcriptome.
- These findings suggest that the duodenum adapts when exposed to Westernized diet offering a pathophysiological linkage to the development of systemic insulin resistance. Conversely, this may also offer explantion for the apparent insulin-sensitizing effect observed with duodenal exclusion surgery.

REFERENCES

1. Cherrington et al. GI Endo Clin N Am. 2017; 27:299-311.

DISCLOSURES

This study was supported by Fractyl Laboratories Inc. Soumitra Ghosh is a consultant to Fractyl Laboratories.

FRACTYL