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## Background

↑ Incidence of **Early-Onset Colorectal Cancer (EOCRC)** in individuals <50 years old + ↑ Incidence of **obesity** in developing countries

Is there a link between **EOCRC** and **obesity**?

- Inflammation is involved in the pathophysiology of both EOCRC & obesity.
- Tumor Associated Macrophages (TAMs) & obesity-related hormones, leptin and adiponectin mediate inflammation.
- TAMs are part of the tumor microenvironment and can switch between a proinflammatory (M1) & anti-inflammatory (M2) phenotype.
- Aconitate Decarboxylase 1 (ACOD1) is an enzyme that produces itaconate from aconitate in the Tricarboxylic Acid Cycle.
- Itaconate is a macrophage-specific metabolite produced by certain macrophage subtypes and has carcinogenic effects.
- M2-like macrophages are associated with tumor progression and worse prognosis.
- The effects of obesity-related hormones and itaconate on the cellular metabolism in EOCRC is unknown.

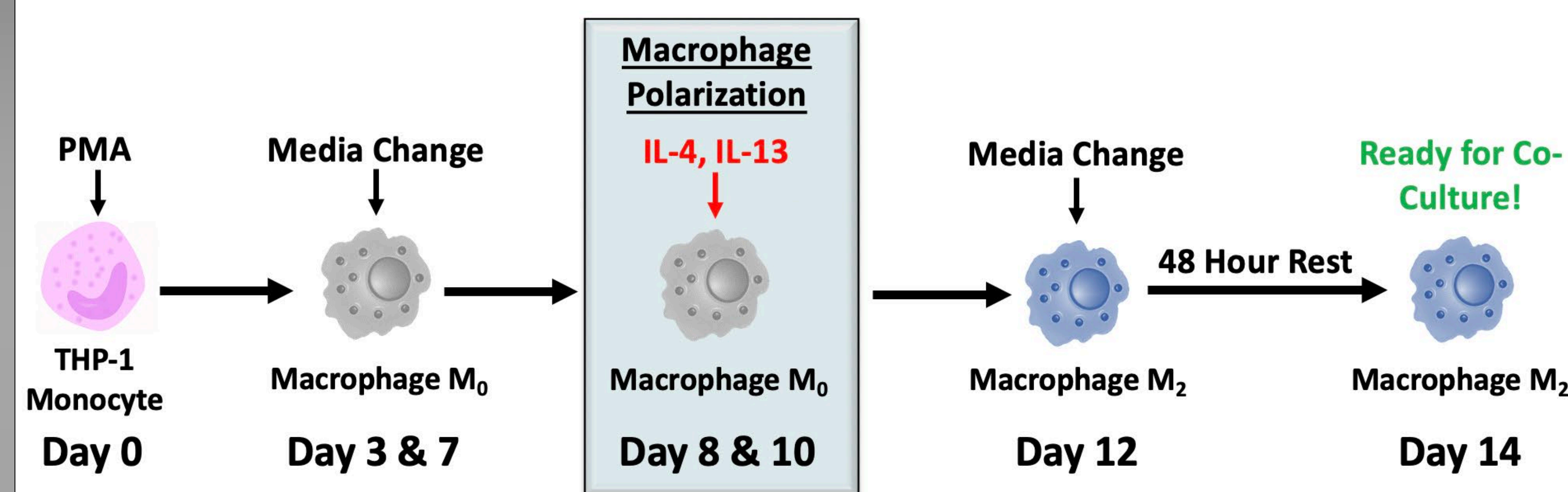
## Aim

The aim of this study was to investigate inflammatory responses in both TAMs and colon cancer cells using an in vitro co-culture model.

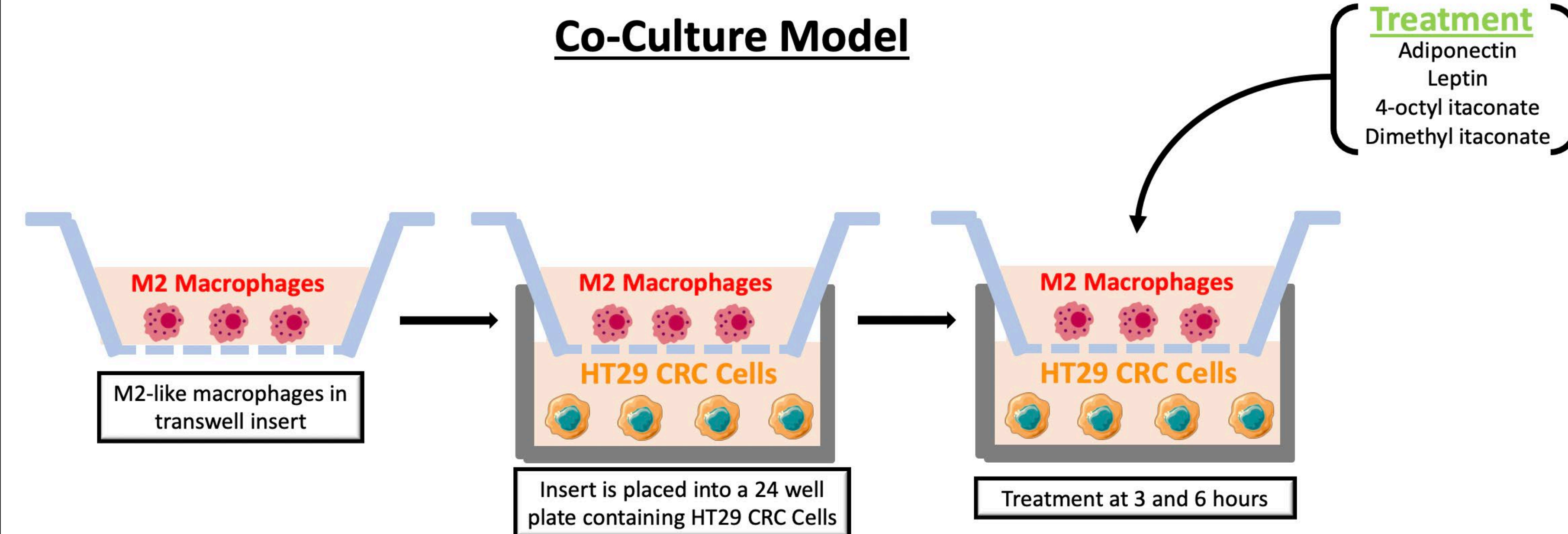
## Methods

- The human monocyte and colon adenocarcinoma cell lines THP-1 and HT29 were acquired (ATCC®, Manassas, VA).
- THP-1 cells were plated into transwell inserts at a concentration of 200,000 cells/insert and polarized into M2-like macrophages within 14-days using phorbol 12-myristate 13-acetate (PMA), interleukin-4 (IL-4) and IL-13.
- M2-like macrophages were then co-cultured with HT29 cells for 24 hours.
- Co-cultured cells were then treated with either leptin, adiponectin, or one of 2 itaconate metabolites: 4-octyl itaconate (OI) or dimethyl itaconate (DI), for 3 and 6 hours.

## Methods



## Co-Culture Model



- Following treatment, total RNA was extracted with mRNeasy Mini Kits (Qiagen®, Germany).
- Reverse transcription was performed using MultiScribe™ Reverse Transcriptase (Invitrogen™, Carlsbad, CA).
- PCR was performed with specific TaqMan gene expression assays for select pro-inflammatory cytokines and ACOD1 (Life Technologies, Carlsbad, CA).

## Results

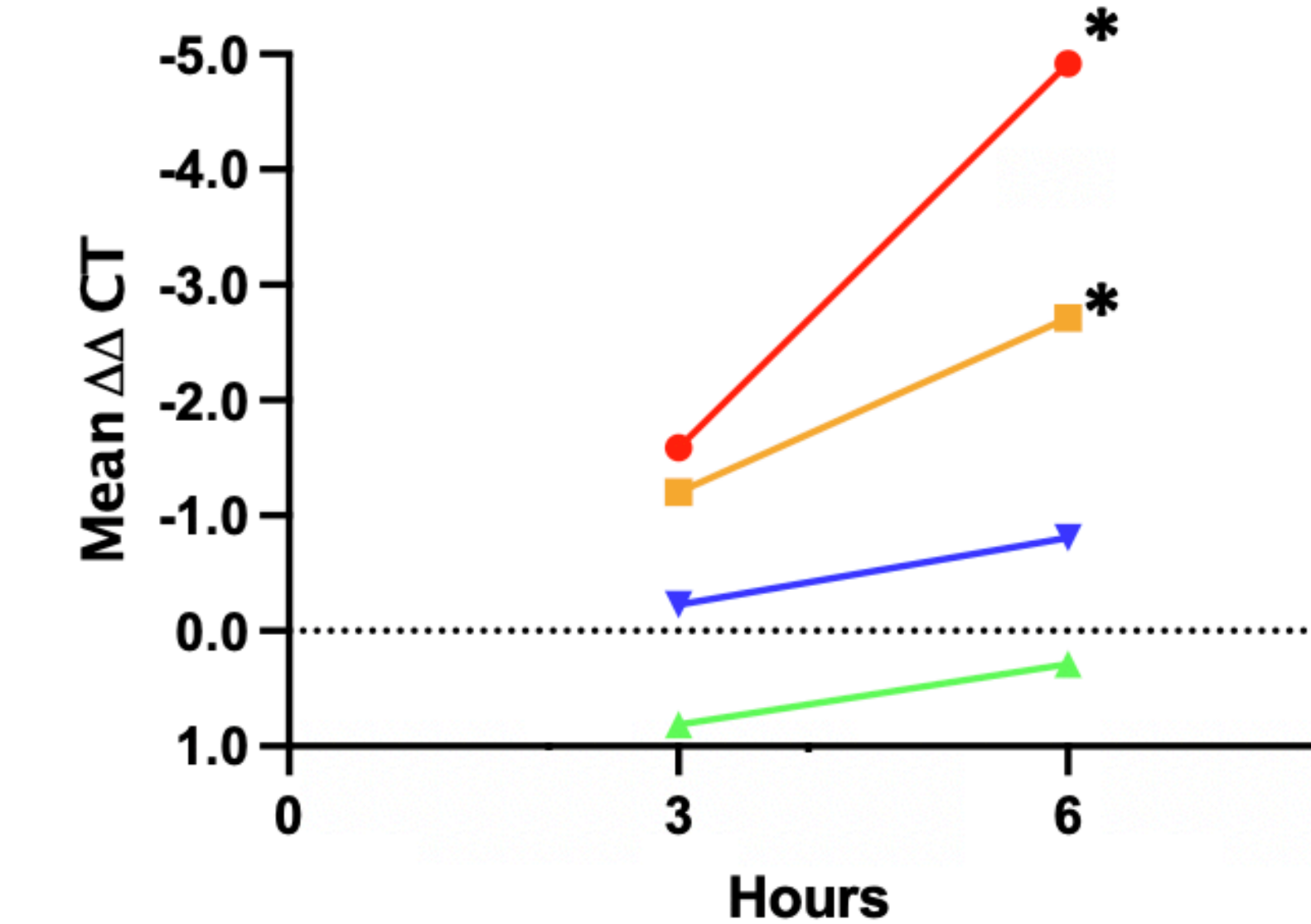
- In M2-like macrophages, proinflammatory IL-1 $\beta$  expression was significantly upregulated following adiponectin (30-fold, p=0.014) and leptin treatment (6-fold, p=0.026) for 6 hours (Fig. 1A).
- Expression of ACOD1 in M2-like macrophages was upregulated with adiponectin treatment (50-fold, p=0.002) for 6 hours (Fig. 1B).
- In HT29 cells, OI treatment resulted in decreased expression of the proinflammatory cytokine CXCL10 at 3 hours (-5 fold-regulation, p=0.045) (Fig. 1C).

## Acknowledgements

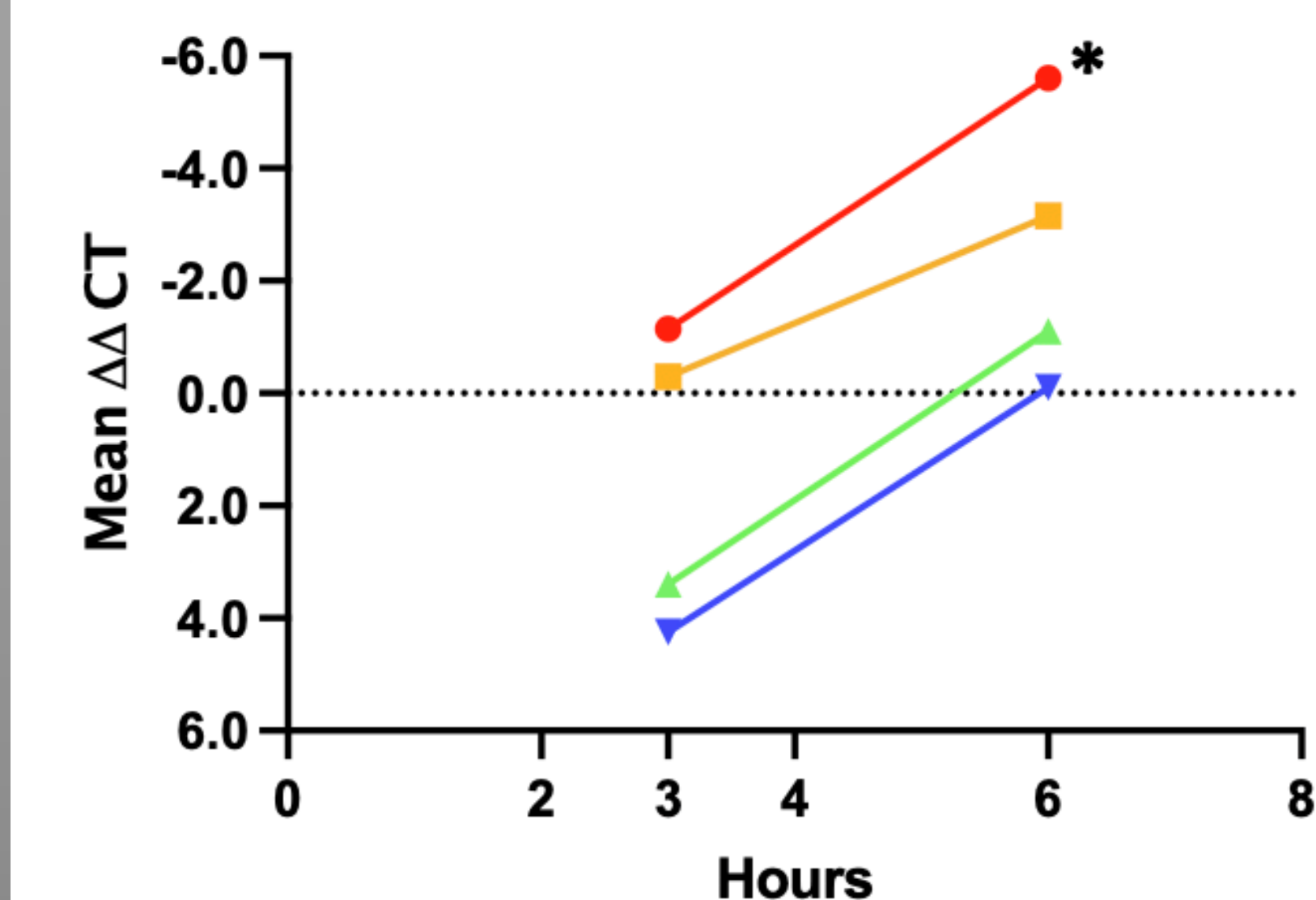
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## Results

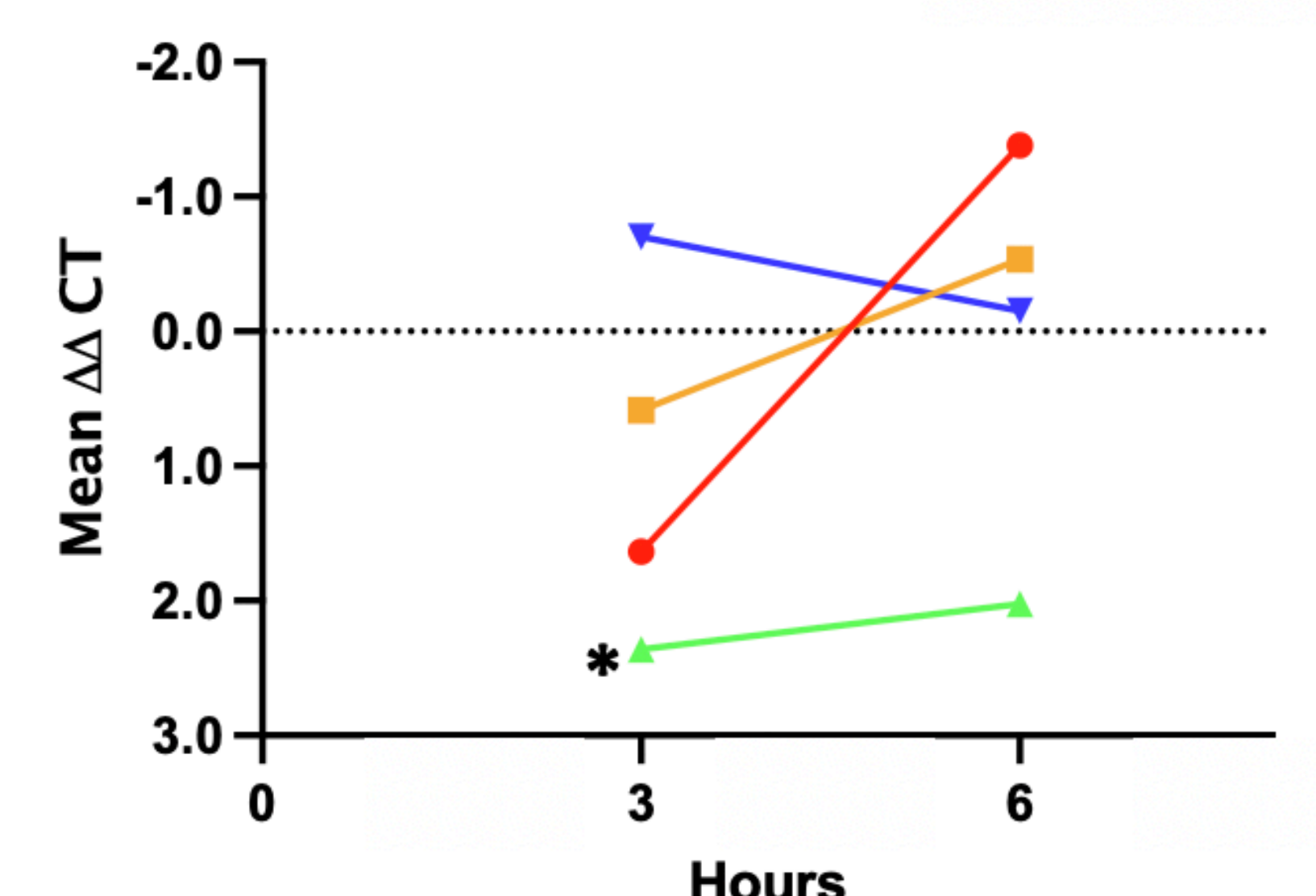
### 1A) IL-1 $\beta$ Expression: THP-1 Cells



### 1B) ACOD1 Expression: THP-1 Cells



### 1C) CXCL10 Expression: HT29 CRC Cells



Legend: Adiponectin (red circle), Leptin (orange square), 4-octyl Itaconate (green triangle), Dimethyl Itaconate (blue inverted triangle)

\*p<0.05, N=2, Mean  $\Delta\Delta$ CT (Figures 1A-1C)

## Conclusion

- Adiponectin and leptin induce cytokine gene expression and itaconate production in TAMs that promote carcinogenic mechanisms in CRC.
- The effects of obesity-related hormones on macrophage polarization and cytokine expression may provide a link between obesity and EOCRC.

## Future Endeavors

- Increase sample size.
- Determine if macrophage specific metabolite itaconate interacts with HT29 cells in co-culture.
- Measure HT29 cell counts before and after co-culture model to determine effects on cellular proliferation.
- Replace HT29 cell line with a different colon cancer cell line in the co-culture model.