Are PUFAs the Missing Link to Treat Cardiovascular Diseases? An Analysis of Egr-1 Gene Expression & Neutral Lipid Droplets in PUFA-Treated Monocytes

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Introduction

- Persistent inflammation contributes to the development of cardiovascular and other chronic diseases
- Often, the consumption and metabolism of certain fatty acids (FA) - like **saturated fatty acids** or **trans-fats** - exasperates inflammation, and accelerates the development of chronic cardiovascular diseases
  - Interestingly, immune cells like monocytes seem aid in chronic disease progression – they contribute to inflammation when they are dysregulated
- Whereas **polyunsaturated fatty acids** – such as alpha-linoleic acid (ALA) – could potentially moderate chronic disease progression by reducing the quantity of circulating triglycerides, cholesterol, and inflammatory markers
- Previous unpublished data has shown that ALA specifically seems to act on monocytes, in addition to upregulating early growth response 1 (Egr-1) in circulating cells
  - (1) Egr-1 suppresses inflammatory gene expression in monocytes (PMID: 33523892) so it may be partly responsible for the previously published anti-inflammatory effects of ALA; (2) Egr-1 promotes fat storage in adipocytes (PMID: 25814662).

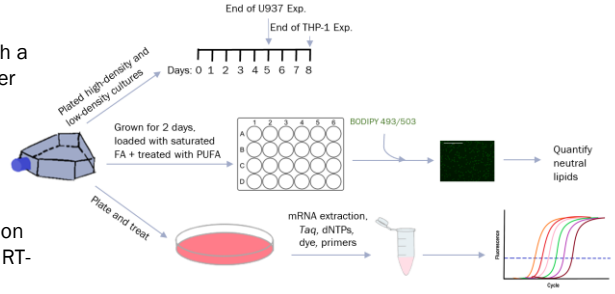
Hypotheses

H_1: ALA in human monocytes will increase Egr-1 expression, compared to other fatty acids like docosahexanoic acid (DHA), eicosapentaenoic acid (EPA), and oleic acid (OA)

H_2: ALA will also increase neutral lipid concentrations in human monocytes, compared to DHA, EPA, OA, and linoleic acid (LA) in monocytes loaded with saturated FAs

Methods

- Cell growth experiments conducted with a hemocytometer
- Neutral lipid experiments analyzed with confocal microscopy
- Egr-1 expression quantified via RT-qPCR



Preliminary Results

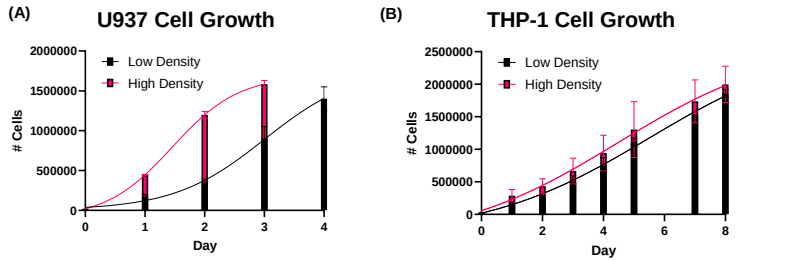


Figure 1. Measurement of monocyte cell line growth curves. Cells were plated with RPMI, 10% fetal bovine serum, and 1% penicillin streptomycin in 37°C and 5% CO_2. (A). Cell growth curve of U937 cells (low density is 100 000 cells/mL, high density is 200 000 cells/mL) (B). Cell Growth Curve of THP-1 cells (low density is 200 000 cells/mL, high density is 300 000 cells/mL).

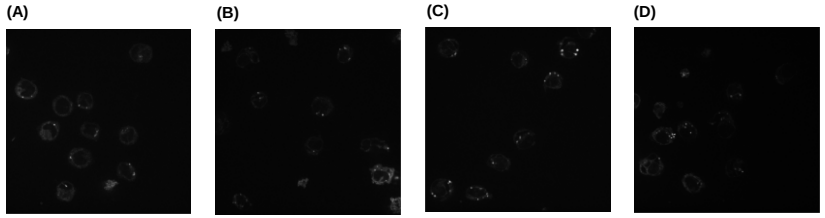


Figure 2. Images of THP-1 monocytes stained with BODIPY 493/503 highlighting neutral lipid droplets. Images with taken using confocal microscopy. (A) Vehicle (ethanol) and 30 µM oleic aid (B) 200 µM palmitic acid (PA) and 30 µM oleic acid (C) 200 µM PA and 30 µM alpha-linoleic acid (D) 200 µM PA and 30 µM docosahexaenoic acid.

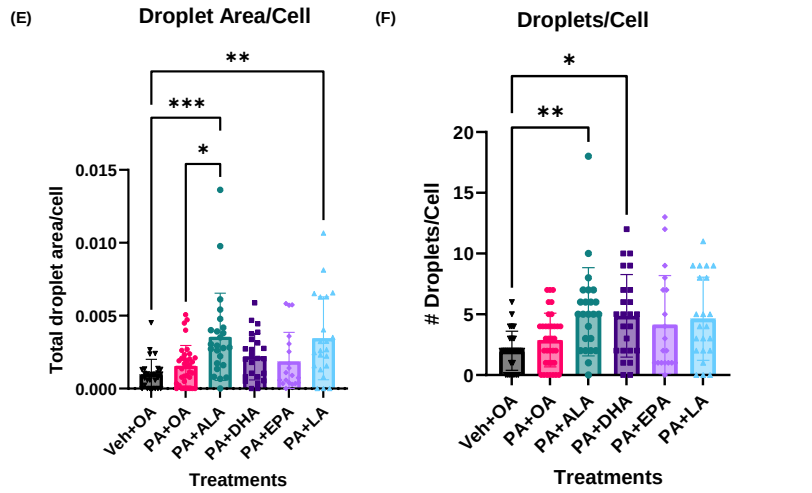


Figure 2. Analysis of neutral lipid droplets in monocytes loaded with 200 µM PA to provide a lipid burden then co-treated with various fatty acids. Neutral lipids were measured using ImageJ software (E). Total droplet area per cell in THP-1 cells treated with 30 µM fatty acid for 24 hours (F) Total number of droplets per cell in THP-1 cells were treated with either 30 µM OA (control), ALA, DHA, EPA, or LA for 24 hours.

Preliminary Results

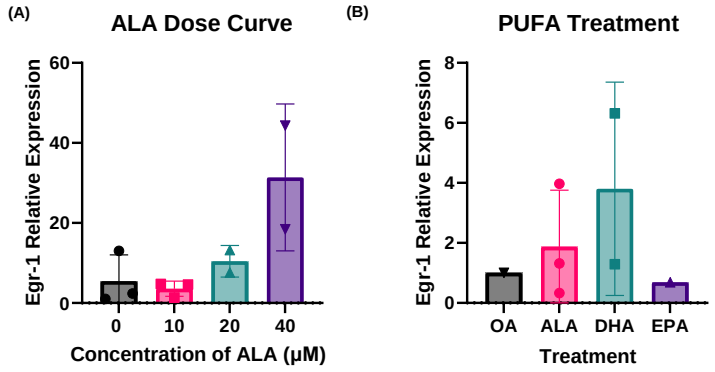


Figure 3. qPCR analysis of Egr-1 expression in THP-1 cells treated with various fatty acids. Egr-1 was measured by the ΔΔCT method using QuantStudio6 flex instrument with GAPDH and beta-actin as reference genes. (A) ALA Dose Curve monocytes were treated with micromolar concentrations of ALA for 24 hours. (B) Egr-1 expression in THP-1 monocytes following treatment of either 40 µM oleic acid (control), ALA, DHA, and EPA for 24 hours.

Discussion

- THP-1 monocytes were a more suitable model than U937 monocytesTotal droplet area per cell was significantly increased in monocytes treated with ALA and LA, compared to the vehicle
- Preliminary results suggest that ALA treatment, compared to the control, may promote increased PA storage
  - Additionally, droplet numbers may also be increased by ALA and DHA – but the comparison to PA-loaded control cells did not reach significance
- Increasing the concentration of ALA tends to trigger an increase in Egr-1 expression concurrently
- Both ALA and DHA may increase Egr-1 expression in monocytes, compared to the vehicle – however more experimental replicates are needed

Future Work

- Neutral lipid and qPCR experiments should be repeated
- Examine the impact of ALA in adipocytes
- Compare WT cells and Egr-1 deficient cells treated with ALA and measure if Egr-1 expression is responsible for the increase in triglyceride storage

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