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-linolenic 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- Previous unpublished data has shown that ALA specifically seems to act on monocytes, in addition to upregulating early growth response 1 (Egr-1) in circulating cells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

H1: ALA in human monocytes will increase Egr-1 expression, compared to other fatty acids like docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and oleic acid (OA)

H2: 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. (A) Cell growth curve of U937 cells (low density is 600 000 cells/mL, high density is 300 000 cells/mL) (B). U937 Cell Growth Curve of THP-1 cells (low density is 62 000 cells/mL, high density is 31 000 cells/mL). Figure 2. Images of THP-1 monocytes loaded with various fatty acids. Neutral lipids were measured using Oil Red O staining and an inverted microscope. Images were taken using standard microscopic conditions. Figure 3. qPCR analysis of Egr-1 expression in THP-1 monocytes following treatment of either 30 μM PA and 30 μM oleic acid (control), ALA, DHA, and EPA for 24 hours. We gratefully acknowledge the funding support from the Rady Faculty of Health Sciences and the College of Pharmacy at the University of Manitoba

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

Discussion
- THP-1 monocytes were a more suitable model than U937 monocytes
- Total 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

Figure 4. Droplet Area/Cell and Droplets/Cell of THP-1 monocytes treated with various fatty acids. Neutral lipids were measured using Oil Red O staining and an inverted microscope. Images were taken using standard microscopic conditions. Figure 5. ALA dose response of Egr-1 expression in THP-1 cells treated with various fatty acids. Egr-1 was measured by the QRT-PCR method using Taqman® probe and SYBR® Green I dye. The ALA dose was 30 μM. We gratefully acknowledge the funding support from the Rady Faculty of Health Sciences and the College of Pharmacy at the University of Manitoba