CHRD 2024: Abstract Submission Form

Presenter Name
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Presenter Status
Masters Student

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Research Category
Basic Science

Title
Omega-3 fatty acids modify monocyte energy metabolism through mitochondrial bioenergetic rewiring

Background
Chronic inflammation is a driving factor in metabolic diseases like obesity and type 2 diabetes. This heightened immune activation, spearheaded by innate immune cells such as monocytes, has been associated with enhanced glucose metabolism, including oxidative phosphorylation. A recent clinical trial showed that supplementation with the omega-3 fatty acid α-linolenic acid (ALA) reduced oxidative phosphorylation rates in circulating monocytes from women with obesity. However, the mechanism(s) remain unknown.

Objective
Therefore, our objective was to replicate the findings in a cell culture model to explore the molecular mechanism.

Methods
THP-1 monocytes were treated for 48h with 10-40 μM of fatty acid, with a bolus dose at 24h. The Seahorse XFe24 and Oroboros O2k Oxygraph instruments were used to approximate catabolic rates (oxidative phosphorylation and glycolysis) with either glucose or palmitic acid provided as a metabolic substrate. Pro-inflammatory cytokine (IL-1□) level was measured by ELISA. Finally, gene expression was assessed by reverse-transcriptase quantitative polymerase chain reaction.

Results
ALA reduced mitochondrial ATP production by ~24% and increased glycolytic ATP production by ~62% in the presence of glucose. ALA also decreased fatty acid oxidation to a similar extent. Unexpectedly, another omega-3 fatty acid, docosahexaenoic acid (DHA) had similar effects on glucose catabolism. Both ALA and DHA treatment reduced IL-1B levels compared to vehicle, ~63% and 42%, respectively. Finally, we identified pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that inhibits the conversion of pyruvate to acetyl-CoA, as a possible mechanistic candidate. It was significantly upregulated by DHA (~7-fold), though only slightly by ALA (~1.4 fold).

Conclusion
Overall, ALA and DHA similarly dampened oxidative phosphorylation rates and suppressed pro-inflammatory cytokine production. Although only DHA significantly elevated PDK4 levels. This is an important step towards understanding how intervention strategies with omega-3 fatty acids could help treat or prevent chronic metabolic diseases relevant to children and youth.

Do you have a table/figure to upload?
Yes

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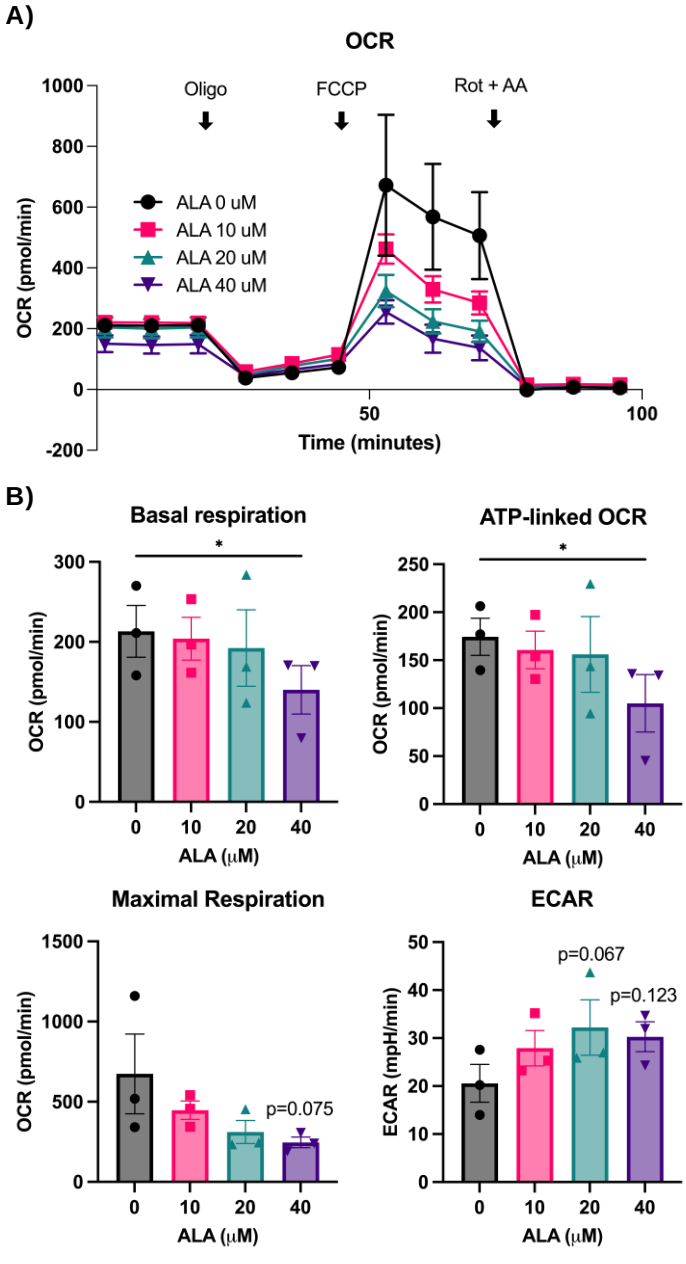

Figure 1. ALA alters bioenergetic parameters of THP-1 cells in a dose-dependent manner. Human THP-1 monocytes were seeded in 6-well cell culture plates at a density of 3.75-4.25x10^5 cells/ml and treated with increasing concentrations of ALA (0 µM, 10 µM, 20 µM, 40 µM) for 48 h with a bolus dose at 24 h. 2x10^5 cells/well were then plated onto 24 well Seahorse plates using the adhesive Celltak (Corning) in complete RPMI XF medium (bicarbonate free). Bioenergetics were assessed by a Seahorse XF Mito Stress Test, performed on a Seahorse XFe24 analyzer. (A) Time plot showing changes in Oxygen consumption rate (OCR) following the injection of Oligomycin (oligo, 1 µM), uncoupling agent (FCCP, 1.5 µM) and Rotenone + Antimycin A (Rot, 0.1 µM + AA, 1 µM). Glucose was used as the metabolic substrate at a concentration of 10 mM. (B) Comparison of Basal respiration, ATP-linked OCR, Maximal respiration, and Extracellular acidification rate (ECAR) between treatments. Data are shown as mean ± SEM of three experimental replicates, where the values from each experiment were averaged from 4-6 technical replicates. Data were analyzed via repeated measures One-Way ANOVA and significance is shown relative to vehicle; * indicates $p < 0.05$.

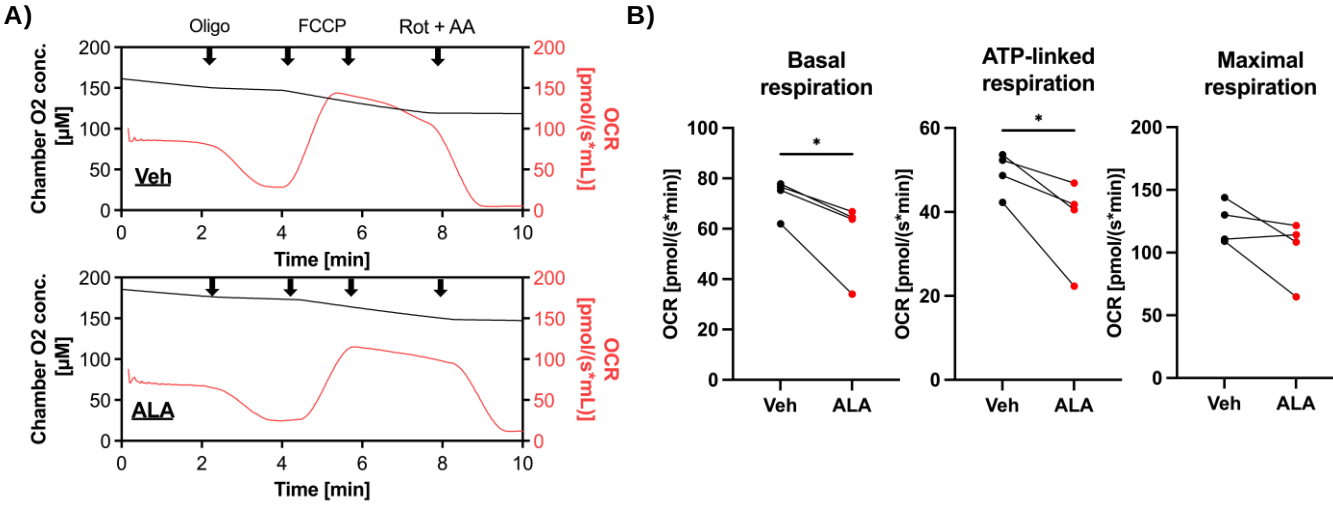

Figure 2. ALA reduces the oxygen consumption rate in monocytes as measured by high-resolution respirometry. THP-1 cells were treated with Vehicle control or 40 µM ALA as in Figure 2, then assessed for oxygen consumption rates using the Oroboros O2k Oxygraph. (A) Time plot showing changes in absolute O_2 concentration (left axis) and oxygen consumption rate (OCR, right axis) for the two conditions, following manual injections of oligomycin (oligo, 2 µM), uncoupling agent (FCCP, 1 µM per injection) and Antimycin A (AA, 2 µM). Glucose was used as the metabolic substrate at a concentration of 11.1 mM in complete RPMI XF medium (bicarbonate free). (B) Comparison of ATP-linked OCR, Basal respiration, and Maximal respiration. Data are shown as mean ± SEM of four experimental replicates, where the values from each experiment were averaged from 4 technical replicates per group. Data were analyzed via paired t-test and significance is shown relative to vehicle; * indicates $p < 0.05$.

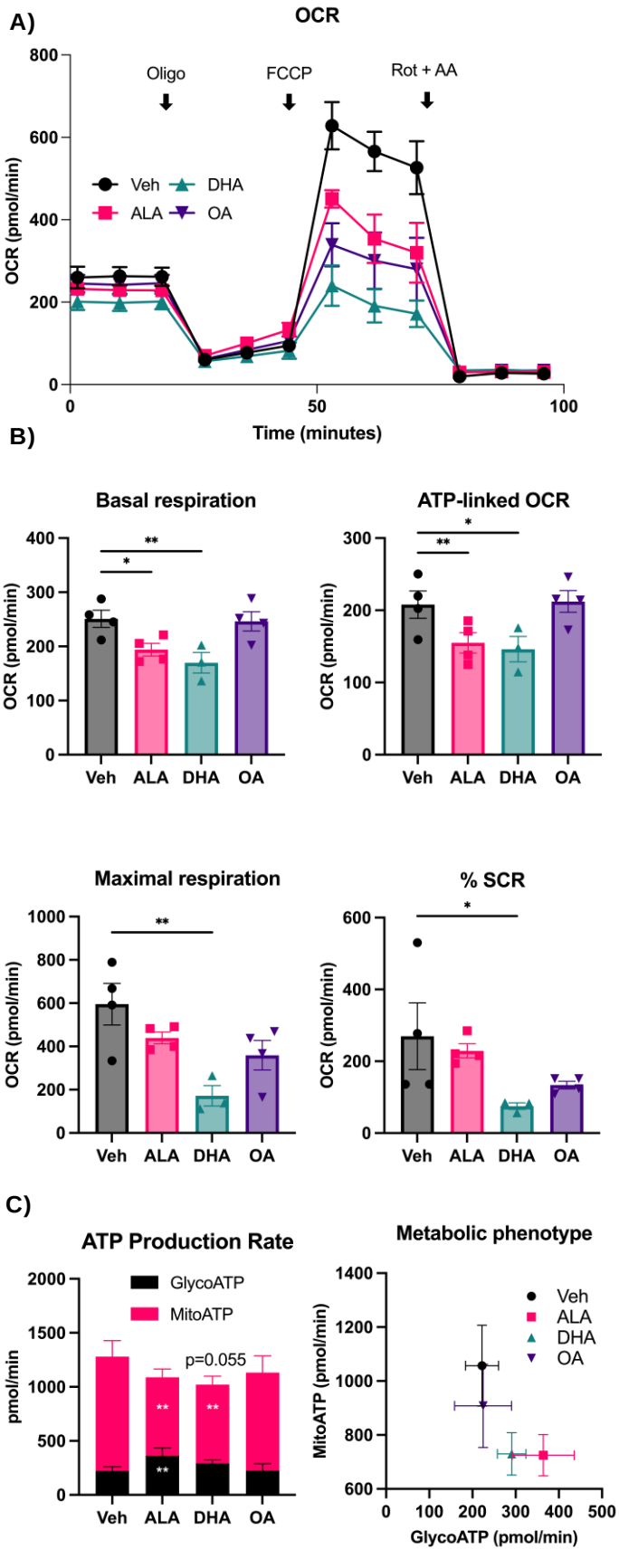

Figure 3. ALA and DHA induce a rewiring of monocyte ATP production. Human THP-1 monocytes were seeded in 6-well cell culture plates at a density of $4.25x10^5$ cells/ml and treated with vehicle or 40 μM of fatty acid (ALA, DHA, OA) for 48 hours with a bolus dose at 24 hours. $2x10^5$ cells/well were then plated onto 24 well Seahorse plates using the adhesive Celltak (Corning) in complete RPMI XF medium (bicarbonate free). A Seahorse XFe24 analyzer was used to assess bioenergetics. (A-B) Time plot of ATP rate Assays showing changes in Oxygen consumption rate (OCR, A) and in Extracellular acidification rate (ECAR), B) following the injection of Oligomycin (oligo, 1 μM), and Rotenone + Antimycin A (Rot, 0.1 μM + AA, 1 μM). Glucose was used as the metabolic substrate at a concentration of 10 mM. (C) Seahorse XF ATP rate Assays were performed to assess relative rates of ATP production via glycolytic (GlycoATP) and mitochondrial (MitoATP) pathways. (D) Graph depicting differences in metabolic phenotype between treatment conditions. Data are shown as mean ± SEM of three experimental replicates, where the values from each experiment were averaged from 4-6 technical replicates per group. Data were analyzed via repeated measures One-Way ANOVA and significance is shown relative to vehicle; * indicates $p < 0.05$, ** indicates $p < 0.01$.

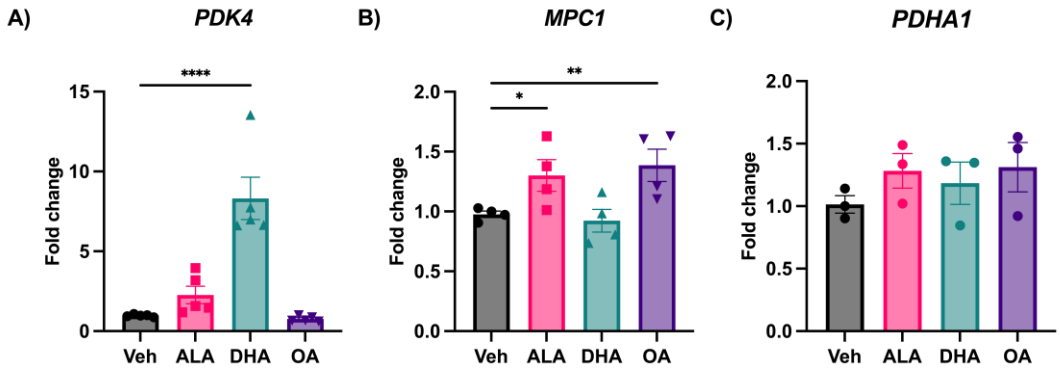

Figure 4. DHA increase PDK4 mRNA levels. THP-1 monocytes were treated with vehicle or 40 μM of fatty acid (ALA, DHA, OA) for 48 hours with a bolus at 24 hours. (A) *pdk4*, (B) *mpc1*, and (C) *pdha1* mRNA levels were assessed by RT-qPCR using the 2^{-ddCt} method with β-actin as the reference gene. Data are shown as mean ± SEM of 3-5 experimental replicates, where the values from each experiment were averaged from 3 technical replicates per group. Data were analyzed via repeated measures One-Way ANOVA and significance is shown relative to vehicle; * indicates $p < 0.05$. Data were analyzed via repeated measures One-Way ANOVA and significance is shown relative to vehicle; * indicates $p < 0.05$, ** indicates $p < 0.01$, **** indicates $p < 0.0001$.

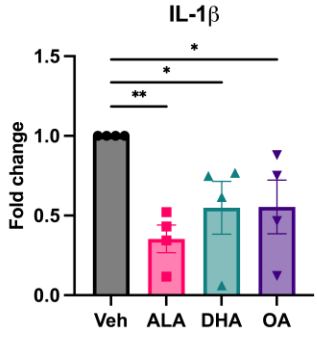

Figure 5. n-3 PUFAs and Oleic acid induce an anti-inflammatory effect on LPS-stimulated THP-1 monocytes. Human THP-1 cells were treated with 40 µM of ALA, DHA, OA, and vehicle in triplicates for 48 h with a bolus at 24 h. 2h prior to supernatant collection, cells were treated with 10ng/ml of LPS (lipopolysaccharide). Data are shown as mean ± SEM of three experimental replicates, where the values from each experiment were averaged from 3 technical replicates per group. Data were analyzed via repeated measures One-Way ANOVA and significance is shown relative to vehicle; * indicates $p < 0.05$, ** indicates $p < 0.01$.

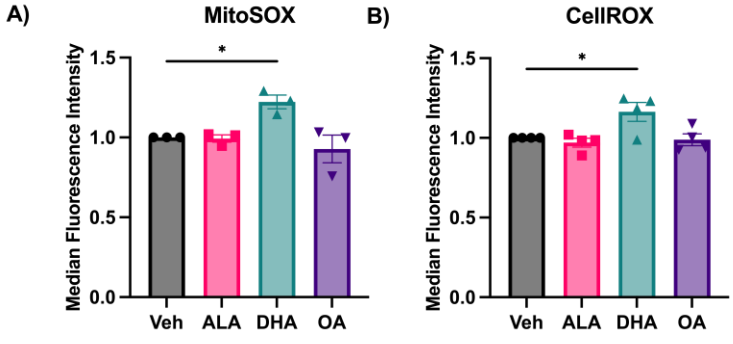

Figure 6. ALA-treated THP-1 cells exhibit no increases in mitochondrial stress. Human THP-1 cells were treated with either vehicle or 40 μM of n-3 FAs (ALA, DHA, OA) for 48 hours with a bolus at 24 hours. Flow cytometry was used to evaluate fluorescence levels indicating either: **A)** Mitochondrial superoxide production using the mitoSOX fluorescent dye; **B)** Cellular reactive oxygen species (ROS) production using the cellROX fluorescent dye. Data are shown as the relative median ± SEM of 3-4 experimental replicate to the vehicle, where the values are from 4 technical replicates per group. Data were analyzed repeated measures via One-Way ANOVA, and significance is shown relative to vehicle: * indicates $P < 0.05$, *** indicates $p < 0.001$.