DHA but not EPA induces the trans-differentiation of C2C12 into white-like adipocytes phenotype

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Introduction

Intramuscular fat can be originated from myoblasts and fibroblasts upon exposure to adipogenic factors. N-3 polyunsaturated fatty acids (PUFAs) supplementations, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are highly recommended during pregnancy because of their neuronal development along with retinal and immune functions improvements. However, Anticipating the potential activation of PPARs by n-3 PUFAs, well-known PPARs' ligands, there is a great chance that the maternal diet enriched with EPA and DHA may induce trans-differentiation of myoblasts into adipocytes. N-3 PUFAs enriched diet is positively correlated with increasing intramuscular fat via up-regulating adipogenesis signature genes. Further, EPA stimulates myoblasts conversion into adipocytes. However, the identity of derived adipocyte is still unknown. We found that concurrent supplementation of EPA and DHA induces C2C12 reprograming into white adipocyte-like cells. Our primary focus here is to determine the independent roles of EPA and DHA on the potential whitening or browning of C2C12 myoblast.

Materials and Methods

C2C12 cell culture and fatty acid (FA) treatment

- Confluent cells were then treated with a white (WDIM) and brown adipogenic differentiation induction medium (BDIM) in the presence or absence of 50μM EPA or DHA.

Oil Red O(ORO) staining

- Cells were fixed with paraformaldehyde 10% for 30 minutes at room temperature followed by 3X washing with PBS. Lipid droplets were stained with ORO for 30 minutes at room temperature.

Real-time PCR

- Total RNA was extracted using RNeasy mini Kit. cDNA was synthesized using X iScript kit. Real-time PCR was performed using CFX Connect Real-Time PCR Detection System. Primers used were designed in accordance with NCBI database and IDT. com.

Western blot assay

- Protein was extracted using lysis buffer, T-PER. samples were separated on precast gels and transferred onto PVDF membrane. The membrane was incubated with primary antibodies overnight and then with secondary antibody for 1 hour. The bands were visualized using ECL immunoblotting clarity system and detected on ChemiDoc TM Touch imaging system.

Oxygen consumption measurement

- Mitochondrial function was evaluated by directly measuring oxygen consumption rate (OCR) using Seahorse XFP. C2C12 cells were seeded in XF plates, and induced to browning. Oligomycin, FCCP, and rotenone/ antimycin-A, were orderly injected at final concentrations of 2μM, 0.7 nM, and 1 μM respectively. The Seahorse XF Cell Mito Stress Test Report Generator was used to analyze the data. The results were normalized to protein concentration.

Results

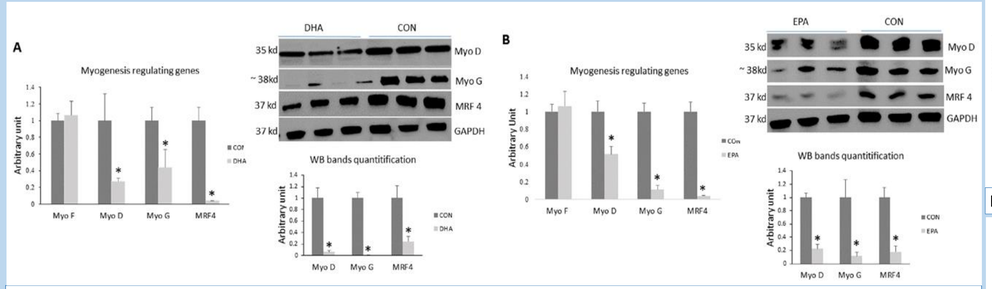

Figure 1 Effect of isolated doses of EPA and DHA on myogenesis regulating genes in C2C12 induced into white adipogenesis

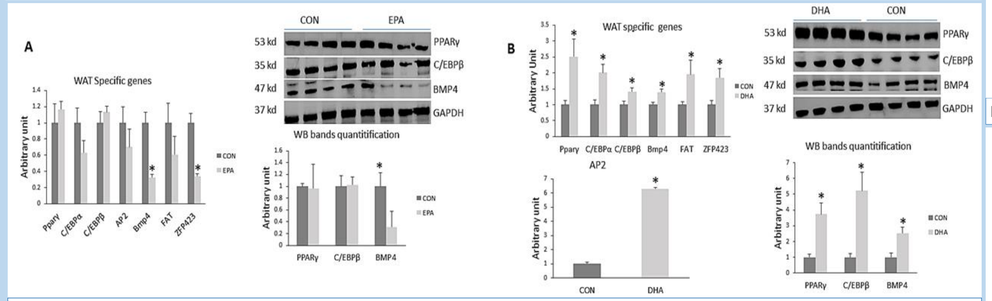

Figure 2 Effect of isolated dosed of EPA and DHA on WAT adipogenesis regulating genes

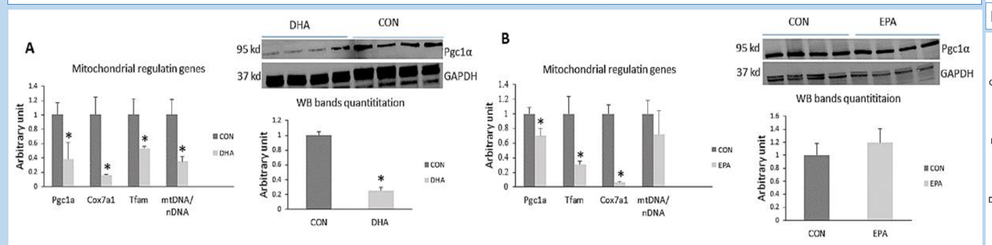

Figure 3 Effect of EPA and DHA on Mitochondrial biogenesis genes

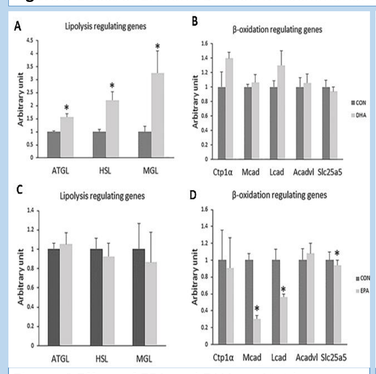

Figure 4 Effect of EPA and DHA on Lipid metabolism reregulating gens

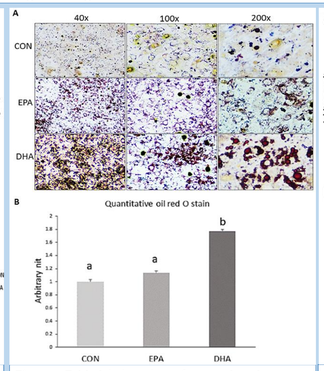

Figure 5 Lipid droplets formation in C2C12 induced into white adipogenesis

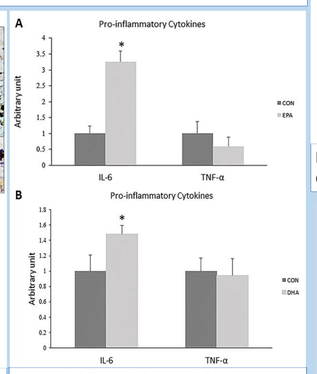

Figure 6 Effect of Epa and DHA on cytokine production regulating genes

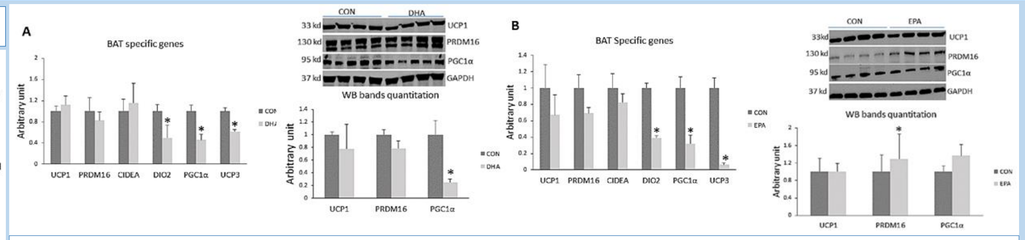

Figure 7 Effect of EPA and DHA on BAT specific genes

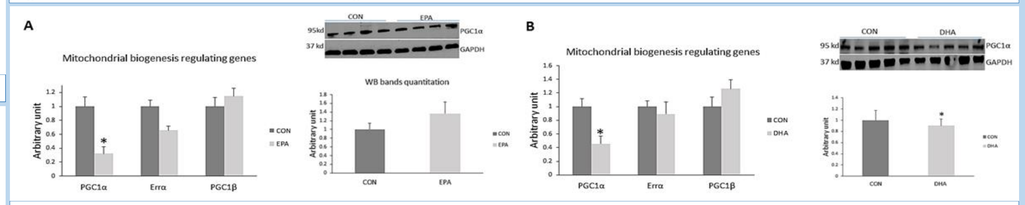

Figure 8 Effect of EPA and DHA on genes regulating mitochondrial biogenesis

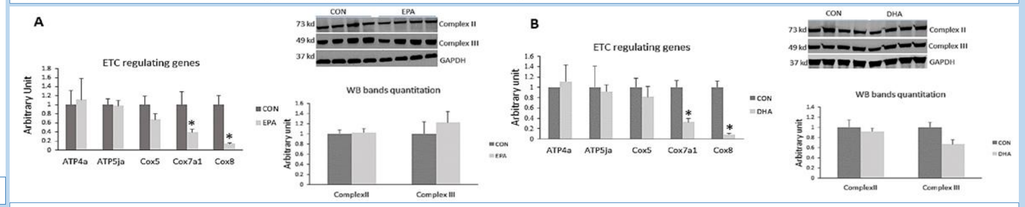

Figure 9 Effect of isolated doses of EPA and DHA on electron transport chain regulating genes

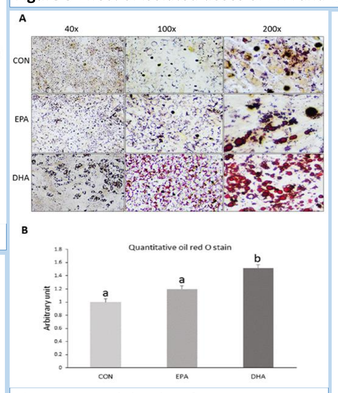

Figure 10 Lipid droplets formation in C2C12 induced into brown adipogenesis

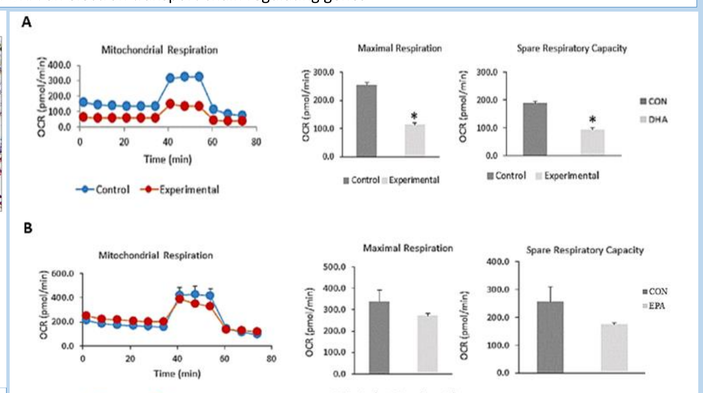

Figure 11 Effect of EPA and DHA on OCR in C2C12 induced to brown adipogenesis

Conclusions

- C2C12 cells losses their myogenic capacity once exposed to either EPA or DHA treatment.
- DHA is an adipogenic factor inducing C2C12 cells trans-differentiate into white-like adipocytes
- Adipocytes derived from C2C12 treated with DHA showed decreased thermogenic capacity independent of UCP1 and reduced mitochondrial copy number and activity
- Adipocytes that arisen from C2C12 after DHA treatment were enriched with big unilocular lipid droplets
- DHA enhances basal lipolysis and fatty acid re-esterification
- EPA is a non-adipogenic factor inhibiting the myogenesis process and acts as an antagonist to the effect of DHA