

ALA, EPA and DHA differentially modulate palmitate-induced lipotoxicity through alterations of its metabolism and storage in C12C12 muscle cells.

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Since few decades, incidence of obesity and type 2 diabetes (T2D) is increasing. Excessive intake of energy leads to fat overload and formation of lipotoxic compounds mainly derived from the saturated fatty acid palmitate in insulin-sensitive tissues (muscle, liver and white adipose tissue), promoting insulin resistance (IR, a well-known metabolic disorder in T2D). Supplementation with n-3 fatty acids (n-3FA) is suggested to reduce lipotoxicity and IR. We hypothesized that, according to the n-3FA used, differential and specific effects on palmitate metabolism in muscle cells will be demonstrated. C2C12 myotubes were treated with 500 μ M of palmitate without or with 50 μ M of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) for 16 hours and collected for measurement of membrane fluidity using diphenyl-hexatriene, ceramide content, insulin-dependent Akt protein phosphorylation (as an index of IR). The assessment of the intracellular metabolism and incorporation of palmitate into lipid fractions (triglycerides, phospholipids, diglycerides) was performed after treatment for 3 hours with [1-¹⁴C]-palmitate. As expected, palmitate-induced IR was restored by EPA and DHA supplementation whereas ALA had no effect compared to palmitate alone. EPA and DHA significantly improved C2C12 membrane fluidity compared to palmitate alone (+8.5% and +13% respectively, $p < 0.05$). Furthermore, palmitate incorporation into the diglyceride fraction was decreased by 31 and 47% by EPA and DHA vs. palmitate, respectively ($p = 0.05$). However, DHA significantly increased the ratio of diglycerides to total lipids vs. palmitate alone ($p < 0.05$), whereas EPA did not. Finally, EPA was more potent to decrease palmitate-induced ceramide accumulation (+174%, $p < 0.05$ vs. control) compared to DHA (-50% and -29% respectively, $p < 0.05$). In conclusion and contrary to ALA, EPA and DHA treatment improved the insulin signalling pathway by differently modulating membrane fluidity and lipid and palmitate metabolism, thus demonstrating that n-3FA have different metabolic impacts on C2C12 lipid metabolism.