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 µM of palmitate without or with 50 µM 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.