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Comparative actions of omega-3 fatty acids on *in-vitro* lipid droplet formation

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ABSTRACT

Storage of fat into lipid droplets (LDs) is the key step in adipogenesis. Previously the omega-3 polyunsaturated fatty acid (n-3PUFA) eicosapentaenoic acid (EPA; C20:5n-3) has been shown to suppress LD formation, yet the actions of other n-3PUFA is unknown. Here, we examined the impact of the three major long chain n-3PUFA; EPA, docosapentaenoic acid (DPA; C22:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) on LD formation in 3T3-L1 adipocytes. Cells were supplemented with 100 μM fatty acid during differentiation. All n-3PUFA significantly reduced LD formation and the metabolic disorder marker, SCD1, in comparison to stearic acid (STA; C18:0). This action was more potent for DHA than either EPA or DPA. Furthermore, DHA significantly increased lipolysis and ATGL gene and protein expression but reduced the gene expression of three proteins related to LD formation (Perilipin A, Caveolin-1 and Cidea), compared with other n-3PUFA. Thus, DHA, above EPA and DPA, markedly suppressed fat storage in LDs in in-vitro adipocytes.

1. Introduction

Adipose tissue is a major endocrine organ that facilitates energy storage and influences aspects of metabolic disease risk [1–3]. Adipocytes primarily store triacylglycerol (TAG) from exogenous fatty acids (FAs) that are trafficked in a central core of lipid droplets (LDs) [4]. Despite this well established route for lipid storage relatively little is understood of how different FAs regulate this pathway.

Adipogenesis is a complex process whereby fibroblasts transitioning into LD-laden adipocytes. This programme of lipid storage is initiated and regulated by key transcription factors, including peroxisome proliferator-activated receptor γ (PPARγ) [5]. The maintenance of LDs is regulated by cell surface lipolytic enzymes; lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) [6–8]. These lipases interact with two key structural proteins within the lipid raft membrane, Perilipin A and Caveolin-1 [9–11]. Caveolin-1 exists in parallel with Perilipin A on LD membranes [12], confirming their roles in LD formation [13,14]. Perilipin A and Caveolin-1 depletion both increase lipolysis [15,16] and suppress LD formation [17–19]. More recently, cell death-inducing DFF45-like effector-A (Cidea), has also been identified to promote LD formation [20], although its exact function remains unknown.

The rate of LD formation and LD size can be influenced by the prevalent FA species [21]. The long chain omega-3 polyunsaturated fatty acids (n-3PUFA), as potent PPARγ regulators, suppress adiposity in rodents [22–24]. Of the n-3PUFA, docosahexaenoic acid (DHA; C22:6n-3) has been shown to suppress adipogenesis in many adipocyte cell lines [24–26] and increase lipolysis by downregulating Perilipin A [27]. Yet eicosapentaenoic acid (EPA; C20:5n-3) suppressed LD formation in an adipocyte cell culture model (3T3-L1) independent of either modifying rate of lipolysis or Perilipin A [28]. Thus this study aims to analyse and compare the actions of EPA and DHA on LD formation and regulation of LD-membrane proteins. Additionally, the effects of docosapentaenoic acid (DPA; C22:5n-3), a major elongated intermediate of EPA conversion to DHA, was examined. Like EPA and DHA, DPA accumulates efficiently in adipose tissue [29], yet its actions are rarely examined independently from either EPA or DHA. DPA may constitute up to 29% of total n-3PUFA intake [30], as it is present not only in fish, but also found in red meat [31]. It is hypothesised that n-3PUFA will suppress LD formation in cultured 3T3-L1 adipocytes, with this action enhanced with increasing chain length and/or degree of unsaturation.

2. Materials and methods

2.1. Chemicals and reagents

Dexamethasone (Dex; D4902), 3-isobutyl-1-methylxanthine (IBMX; I5879), insulin (I5500), low-endotoxin FA-free BSA (A8806),

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EPA (44864), and stearic acid (STA; C18:0) (85679) (FAs were with purity >99%) were obtained from Sigma (St. Louis, MO). DPA and DHA were from Nu-Chek Prep. Inc. (MN, USA). Foetal bovine serum (FBS) and DMEM were purchased from Invitrogen (Carlsbad, CA). FAs were dissolved in 100% ethanol as stock solutions of 100 mM and stored in the dark at −20 °C before use. The Acyl-CoA oxidase-based colourimetric kit (glycerol) was obtained from Wako (Tokyo, Japan). BCA protein assay kit was from Pierce (Rockford, USA). Anti-SCD1 (M38) antibody was from Cell Signaling Technology (Genesearch Pty Ltd., Queensland, Australia), anti-ATGL antibody was purchased from Sapphire Bioscience (NSW, Australia), while anti-tubulin antibody was from SantaCruz (CA, USA). Secondary HRP-conjugated mouse

Table 1
Gene specific forward and reverse primer oligonucleotide sequences used for RT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	NCBI accession number
18S	GTTGGTGGAGCGATTTGTCTGG	AGGGCAGGGACTTAATCAACGC	NR_003278.1
28S	TGGGAATGCAGCCCAAAG	CCTTACGGTACTTGTTGGCTATCG	NR_003279.1
ATGL	GAGCCCCGGGGTGGAACAAGAT	AAAAGGTGGTGGGCAGGAGTAAGG	NM_025802
Caveolin-1	GCACACCAAGGAGATTGACC	GAATGGCAAAGTAAATGCCC	NM_007616.3
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG	NM_007702
Cyclophilin	TCTGCTGTCTTTGGAACTTTGTC	CTGATGGCGAGCCCTTG	NM_008907
FADS2	AGTCGACATGGGGAAGGGAGGTAACCAG	TCATTTATGGAGGTAAGCATC	NM_019699
SCD1	ATGCCGGCCCACATGCTCCAA	TCAGCTACTCTTGTGACTCCC	NM_009127
HSL	CCTACTGCTGGGCTGTCAA	CCATCTCGCACCCTCACT	NM_010719
LPL	AGTAGACTGGTTGTATCGGG	AGCGTCATCAGGAGAAAGG	NM_008509
PPARγ	GGAATGGGAGTGGTCATCCA	CCCACCAACTTCGGAATC	NM_001127330
Perilipin A	TGCTGGATGGAGACCTC	ACCGGCTCCATGCTCCA	NM_001113471

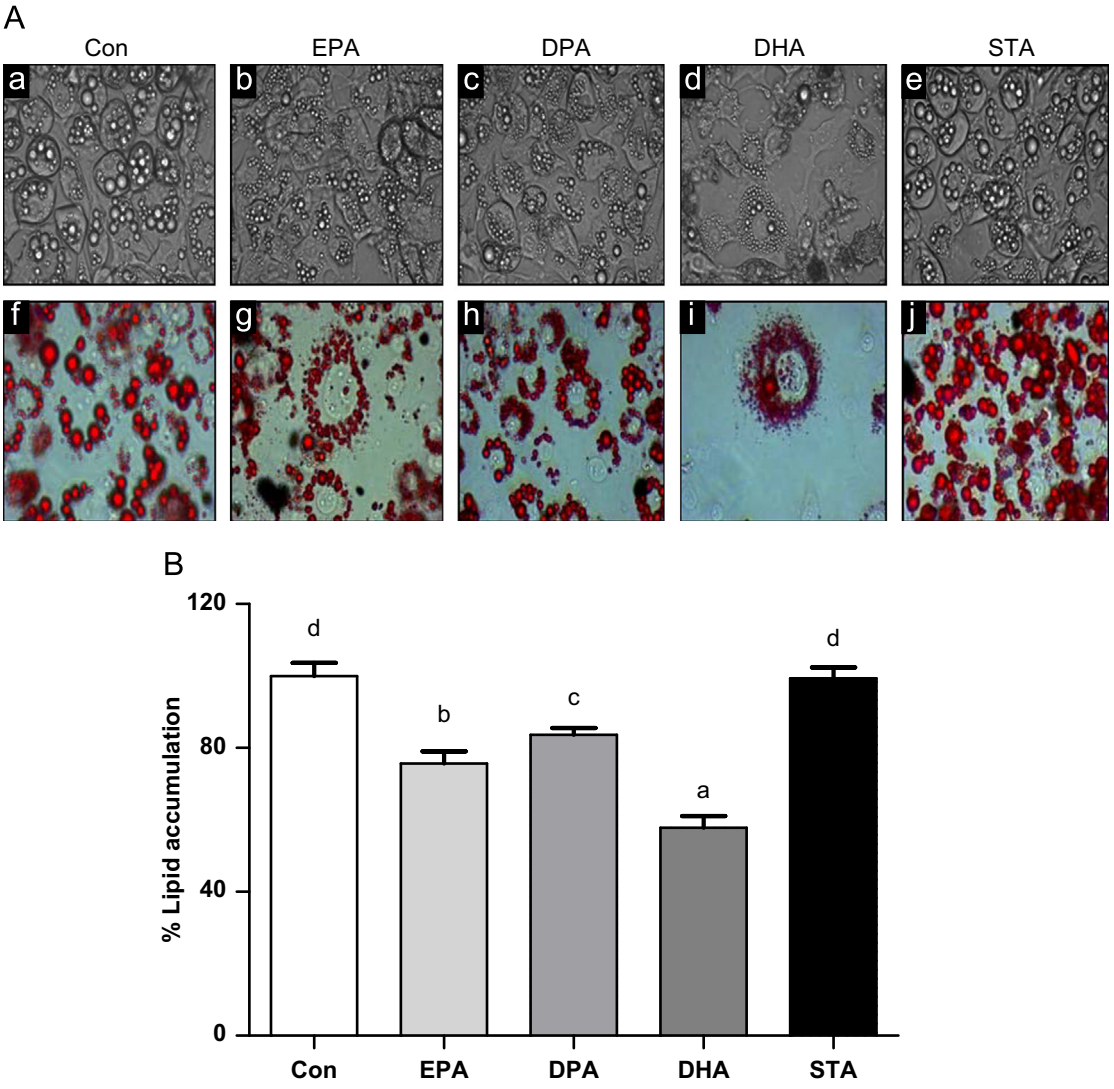

Fig. 1. Effects of EPA, DPA, DHA, and STA on adipocyte morphology and lipid accumulation. FAs were included in adipogenic media throughout differentiation. (A) Cell morphology were analysed before (a–e) or after (f–j) Oil Red O stain. (B) The stained lipid fraction was measured at $A_{520\ nm}$. Values are expressed as means of per cent lipid accumulation ± SEM ($n=8$) relative to vehicle control (Con), with different superscript letters mean $P<0.05$ by Tukey's *post hoc* tests.