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Fucoxanthin exerts differing effects on 3T3-L1 cells according to differentiation stage and inhibits glucose uptake in mature adipocytes

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ABSTRACT

Progression of 3T3-L1 preadipocyte differentiation is divided into early (days 0–2, D0–D2), intermediate (days 2–4, D2–D4), and late stages (day 4 onwards, D4-). In this study, we investigated the effects of fucoxanthin, isolated from the edible brown seaweed *Petalonia binghamiae*, on adipogenesis during the three differentiation stages of 3T3-L1 preadipocytes. When fucoxanthin was applied during the early stage of differentiation (D0–D2), it promoted 3T3-L1 adipocyte differentiation, as evidenced by increased triglyceride accumulation. At the molecular level, fucoxanthin increased protein expression of peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα), sterol regulatory element-binding protein 1c (SREBP1c), and aP2, and adiponectin mRNA expression, in a dose-dependent manner. However, it reduced the expression of PPARγ, C/EBPα, and SREBP1c during the intermediate (D2–D4) and late stages (D4–D7) of differentiation. It also inhibited the uptake of glucose in mature 3T3-L1 adipocytes by reducing the phosphorylation of insulin receptor substrate 1 (IRS-1). These results suggest that fucoxanthin exerts differing effects on 3T3-L1 cells of different differentiation stages and inhibits glucose uptake in mature adipocytes.

1. Introduction

Obesity is characterized by excessive fat deposition, associated with morphological and functional changes in adipocytes [1]. Studies of adipose tissue biology have led to an improved understanding of the mechanisms that link metabolic disorders with altered adipocyte function [2]. The study of adipogenesis has been greatly facilitated by the establishment of immortal preadipocyte cell lines, such as 3T3-L1 preadipocytes. The adipocytes generated from these cells exhibit most of the key features of adipocytes *in vivo* [3].

Adipogenesis is characterized by morphological changes, growth arrest (day 0, D0), and clonal expansion (day 0–2, D0–D2) in adipose cells, followed by a complex sequence of changes in gene expression and lipid storage [4]. The master adipogenic transcriptional regulators are members of the CCAAT/enhancer-binding protein (C/EBP) family and peroxidase proliferator-activated receptor γ (PPARγ). These factors regulate adipocyte differentiation by modulating the expression of their target genes in a coordinated fashion [5–8].

Recent investigations suggest that sterol-regulatory element binding protein 1c (SREBP1c) is the earliest transcription factor involved in adipocyte differentiation [9]. It acts to induce the expression of C/EBPα, PPARγ, and SREBP1c [10–12]. C/EBPα and PPARγ, in turn, promote terminal differentiation, by activating transcription of the gene encoding the fatty acid-binding protein aP2, which is involved in establishing and maintaining the adipocyte phenotype. SREBP1c increases the expression of many lipogenic genes, including fatty acid synthase. Loss-of-function studies have shown that PPARγ is necessary and sufficient to promote adipogenesis [13,14], and that C/EBPα is influential in maintaining the expression of PPARγ [15].

It is believed that the ingestion of edible brown seaweeds is beneficial to human health. Fucoxanthin (Fig. 1A), a major marine carotenoid, has been isolated from brown seaweeds, such as *Undaria pinnatifida*, *Hijikia fusiformis*, and *Sargassum fulvellum*. It has several physiological activities, including anti-cancer [16], anti-carcinogenic [17], anti-inflammatory [18], anti-oxidant [19], and anti-obesity [20,21] effects. Maeda et al. [22] further reported that fucoxanthin, when applied to cells continuously for 5 days (D2–D7), inhibited the differentiation of 3T3-L1 preadipocytes into adipocytes by down-regulating PPARγ.

However, the effects of fucoxanthin on cells at different differentiation stages (early stage, D0–D2; intermediate stage, D2–D4; late

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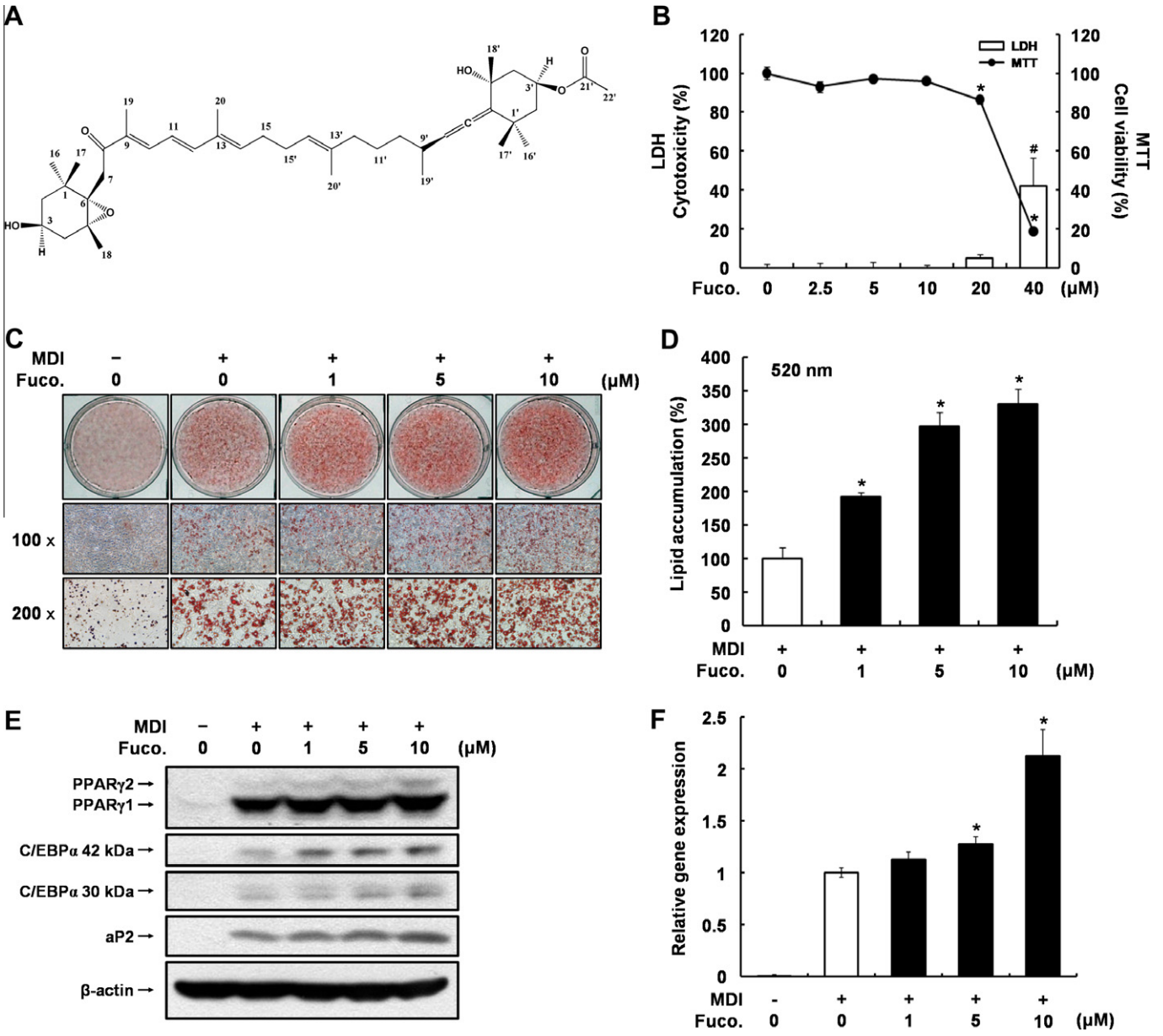

Fig. 1. Effects of fucoxanthin on adipocyte differentiation of 3T3-L1 preadipocytes. Fucoxanthin was isolated by semi-preparative HPLC and identified based on its ^{13}C NMR spectra. (A) Chemical structure of fucoxanthin isolated from *P. binghamiae*. (B) Cytotoxic effects and effects on cell viability in 3T3-L1 preadipocytes. 3T3-L1 preadipocytes were incubated with MDI differentiation medium I for 2 days with or without the indicated concentrations of fucoxanthin. At day 8, the cells were fixed and stained with Oil Red O. Oil Red O-stained adipocytes were photographed at 100× and 200× magnification. (C) The Oil Red O was eluted and quantified at 520 nm. (D) Total protein and total RNA were extracted (at day 6 and day 4, respectively) and the expression of adipocyte-specific proteins and adiponectin mRNA analyzed by Western blotting (E) and real-time PCR (F), respectively. All values are presented as means ± SD ($n = 3$; $^{*}P < 0.05$ and $^{\#}P < 0.05$ compared with no-Fuco control). Fuco, fucoxanthin. The data shown are representative of three independent experiments.

stage, D4–D7) and mature adipocytes have not been reported. In this study, we isolated fucoxanthin from the edible seaweed *Petalonia binghamiae*, extracts of which have anti-diabetic and anti-obesity properties [23,24], and investigated its effects on adipogenesis and glucose uptake in differentiating preadipocytes and fully differentiated adipocytes.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin (PS) were from Gibco BRL (Grand Island, NY, USA). Antibodies to PPARγ, aP2, C/EBPα, insulin receptor substrate 1 (IRS-1), and phospho-Ser473-Akt were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An antibody to SREBP1c was obtained from BD Biosciences (San Jose, CA, USA). Antibodies to Akt and phospho-Ser636/639-IRS-1 were from Cell Signaling Technology (Beverly, MA, USA). Phosphate-buffered saline (pH 7.4; PBS), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma Chemical Co. (St. Louis, MO, USA). A lactate dehydrogenase (LDH) cytotoxicity detection kit was purchased from Takara Shuzo Co. (Otsu, Shiga, Japan). 2-deoxy-D-[^{3}H]glucose was obtained from Amersham Biosciences (Piscataway, NJ, USA). All other reagents were purchased from Sigma Chemical Co. unless otherwise stated.