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Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-A^y mice

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ABSTRACT

Fucoxanthin, a marine carotenoid found in edible brown seaweeds, attenuates white adipose tissue (WAT) weight gain and hyperglycemia in diabetic/obese KK-A^y mice, although it does not affect these parameters in lean C57BL/6J mice. In perigonadal and mesenteric WATs of KK-A^y mice fed fucoxanthin, mRNA expression levels of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α), which are considered to induce insulin resistance, were markedly reduced compared to control mice. In contrast to KK-A^y mice, fucoxanthin did not alter MCP-1 and TNF-α mRNA expression levels in the WAT of lean C57BL/6J mice. Interleukin-6 (IL-6) and plasminogen activator inhibitor-1 mRNA expression levels in WAT were also decreased by fucoxanthin in KK-A^y mice. In differentiating 3T3-F442A adipocytes, fucoxanthinol, which is a fucoxanthin metabolite found in WAT, attenuated TNF-α-induced MCP-1 and IL-6 mRNA overexpression and protein secretion into the culture medium. In addition, fucoxanthinol decreased TNF-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) mRNA expression in RAW264.7 macrophage-like cells stimulated by palmitic acid. These findings indicate that fucoxanthin regulates mRNA expression of inflammatory adipocytokines involved in insulin resistance, iNOS, and COX-2 in WAT and has specific effects on diabetic/obese KK-A^y mice, but not on lean C57BL/6J mice.

Introduction

Obesity has increased drastically in recent years and is currently considered to be a major risk factor for type-2 diabetes, hypertension, and dyslipidemia [1,2]. The cluster of these three diseases is called the metabolic syndrome and its incidence is a worldwide problem [3,4]. The excessive fat accumulation observed in obesity leads to the dysregulation of adipocytokine production in white adipose tissue (WAT),[1] which is closely involved in the development of metabolic syndrome [5–7]. Furthermore, recent studies have indicated that macrophages infiltrate WAT during the development of obesity [8,9], causing a chronic low-grade inflammation characterized by the upregulation of pro-inflammatory adipocytokines, such as tumor necrosis factor α (TNF-α) [10] and downregulation of an anti-inflammatory adiponectin [11]. Suganami et al. reported that saturated fatty acid and TNF-α derived from adipocytes and macrophages, respectively, organize a paracrine loop that lead to inflammation in adipose tissue [12]. The over-production of pro-inflammatory adipocytokines in obese WAT has an important role in the pathogenesis of insulin resistance and obesity-related type-2 diabetes [13,14].

Fucoxanthin is a marine carotenoid found in edible brown seaweeds such as *Undaria pinnatifida* and *Hijikia fusiformis*. Its structure, 5,6-epoxy-3′-ethanoyloxy-3,5′-dihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one, including an allenic bond, epoxide, and acetyl residue, is different from that of other carotenoids such as β-carotene and lutein. We previously reported that dietary fucoxanthin attenuates the weight gain of WAT and improves hyperglycemia in diabetic/obese KK-A^y mice [15]. These findings suggest that fucoxanthin is an effective natural compound for the prevention of obesity and its related type-2 diabetes. Compared to lean C57BL/6J mice, mRNA for monocyte chemoattractant protein-1 (MCP-1) and TNF-α mRNA is overexpressed in WAT of diabetic/obese KK-A^y mice [16]. MCP-1 recruits monocytes to adipose tissue and induces the over-production of inflammatory adipocytokines [17,18]. MCP-1 also inhibits insulin-dependent glucose uptake, leading to insulin resistance [19]. TNF-α and IL-6 are important pro-inflammatory adipocytokines that influence insulin sensitivity [20,21]. Therefore, the suppressive effects of fucoxanthin on the development of obesity and diabetes may

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[1] *Abbreviations used:* WAT, white adipose tissue; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; BAT, brown adipose tissue.

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depend on changes in the production of adipocytokines in WAT. In addition, macrophages produce inflammatory mediators, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2) synthesized by inducible nitric oxide synthase (iNOS) [22] and cyclooxygenase-2 (COX-2) [23]. Recently, iNOS mRNA was reported to be upregulated in obese WAT [24]. To clarify the mechanism underlying the effects of fucoxanthin, we investigated the effects of fucoxanthin on the production of inflammatory mediators in WAT with regard to the interaction between adipocytes and macrophages.

In the present study, we first compared the effects of fucoxanthin on adipose tissue weight and blood glucose levels in diabetic/obese KK-A^y mice and lean C57BL/6J mice. An understanding of how fucoxanthin specifically affects these functions in diabetic/obese mice is very important to clarify the mechanism associated with its anti-obesity and anti-diabetic effects. The regulatory effects of fucoxanthin on the mRNA expression of adipocytokines were then investigated in the WAT from diabetic/obese and lean mice. In addition, we performed a detailed examination of the regulatory effects of fucoxanthin on the mRNA expression of adipocytokines and protein production using a cell culture system of adipocytes and macrophages, respectively. Our findings indicate that fucoxanthin downregulates MCP-1, TNF-α, and IL-6 mRNA in the WAT from diabetic/obese KK-A^y mice, but not from lean C57BL/6J mice. Fucoxanthinol, a metabolite of fucoxanthin, suppressed MCP-1 and IL-6 production in TNF-α-stimulated 3T3-F442A cells, and TNF-α production in palmitic acid-stimulated RAW264.7 cells.

Materials and methods

Materials

Commercial seaweed powder from *U. pinnatifida* was purchased from the market in Hakodate, Japan. Murine preadipocytes 3T3-F442A and the macrophage-like cell line RAW264.7 were obtained from the Dainippon Sumitomo Pharma (Osaka, Japan). Fetal bovine serum (FBS) was purchased from Thermo Trace (Melboume, Australia). Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium were purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan), and Mediatech Inc. (Manassas, VA), respectively. Recombinant murine TNF-α was obtained from BioVision (Mountain View, CA). Palmitic acid, insulin, isobutylmethylxanthine (IBMX), and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO).

Fucoxanthin and fucoxanthinol preparation

Crude lipids containing fucoxanthin were extracted from the commercial seaweed powder using acetone. Fucoxanthin was purified from the extracted crude lipids by silica gel column chromatography with *n*-hexane/acetone (8:2, v/v) as previously described [25]. Fucoxanthinol was prepared from the separated fucoxanthin by hydrolysis with porcine pancreas lipase and purified by high performance liquid chromatography (HPLC, Hitachi High-Technologies Co., Tokyo, Japan)) equipped with an ODS column (250 × 4.6 mm i.d., Nomura Chemical Co. Ltd., Aichi, Japan) according to the previously described procedure [25].

Animal experiments

KK-A^y (female, 4-week-old) and C57BL/6J mice (female, 4-week-old) were obtained from CREA Japan (Tokyo, Japan) and Charles River Japan (Ibaraki, Japan), respectively. The mice had free access to drinking water and were fed a diet prepared according to the recommendations of the American Institute of Nutrition (AIN-93G) at 23 ± 1 °C and 50% humidity with a 12-h light/12-h dark cycle. KK-A^y and C57BL/6J mice were assigned to two groups of six mice, and provided with a control diet (AIN-93G) or experimental diet, in which 0.2% fucoxanthin was added to the control diet, after acclimation to the conditions for 1 week. The composition of the control diet was 20% casein, 39.7% corn starch, 10% sucrose, 13.2% dextrinized cornstarch, 5% cellulose, 7% soybean oil, 3.5% AIN-93G mineral mix, 1% AIN-93G vitamin mix, 0.3% L-cystine, 0.25% choline bitartrate, and 0.0014% *tert*-butylhydroquinone. After feeding with the control and experimental diets for 4 weeks, mice were starved for 12 h and dissected while anesthetized with diethyl ether. Perigonadal and mesenteric WATs and brown adipose tissue (BAT) were removed, weighed, and immediately stored in RNA later™ (Sigma Chemical Co., St. Louis, MO) for quantitative real time PCR analysis. All procedures for the use and care of animals for this research were approved by the Experimental Animal Care and Use Committee of Hokkaido University.

Blood glucose

Blood glucose was determined using a blood glucose monitor, the Glutest Neo Sensor (Sanwa Kagaku Kenkyusho Co. Ltd., Aichi, Japan), without fasting at 1 day before dissection (after 27 days of feeding). This sensor is an amperometric sensor with FAD-dependent glucose dehydrogenase and $Fe(CN_6)^{3-}$.

Histological observation

Small pieces of perigonadal WAT were removed, rinsed with saline, and fixed in 15% formaldehyde/PBS. Sections of formalin-fixed tissue specimens were stained with hematoxylin–eosin and observed under light microscopy. Infiltration of macrophages was detected in the formalin-fixed paraffin-embedded perigonadal WAT by immunohistochemical staining with the anti-F4/80 antibody (1:100) (Serotec Ltd., Oxford, UK).

Cell cultures

3T3-F442A preadipocytes were cultured in DMEM with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cells were cultured to confluence and maintained for additional 24 h. Differentiation of 3T3-F442A was initiated with DMEM containing 10 μg/mL insulin, 0.5 mM IBMX, and 0.1 μM dexamethasone for 48 h. The medium was then replaced with DMEM containing fucoxanthinol, and further incubated changing to fresh medium every 2 days. Fucoxanthinol was added to the culture medium as an ethanol solution. Ethanol concentration was 0.1% in the culture medium without cytotoxicity. After 120 h incubation in the presence of fucoxanthinol, 20 ng/ml TNF-α was added to the culture medium and incubation was continued for an additional 24 h. Then, 3T3-F442A cells were washed twice with PBS and used for total RNA extraction.

RAW264.7 cells (1×10^5 cells/well) were maintained in 24-well plates with RPMI 1640 with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin for 24 h. Fucoxanthinol was added to the culture medium as an ethanol solution, and incubated for another 24 h. Ethanol concentration was 0.1% in the culture medium without cytotoxicity. Further, palmitic acid (0.5 mM) was added into culture medium as an albumin complex, and cells were incubated for an additional 24 h. RAW264.7 cells were washed twice with PBS and used for total RNA extraction.

Quantitative real time RCR

Total RNA was extracted from perigonadal and mesenteric WATs using the RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo,