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Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues ☆

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Abstract

Mitochondrial uncoupling protein 1 (UCP1) is usually expressed only in brown adipose tissue (BAT) and a key molecule for metabolic thermogenesis to avoid an excess of fat accumulation. However, there is little BAT in adult humans. Therefore, UCP1 expression in tissues other than BAT is expected to reduce abdominal fat. Here, we show reduction of abdominal white adipose tissue (WAT) weights in rats and mice by feeding lipids from edible seaweed, *Undaria pinnatifida*. Clear signals of UCP1 protein and mRNA were detected in WAT of mice fed the *Undaria* lipids, although there is little expression of UCP1 in WAT of mice fed control diet. The *Undaria* lipids mainly consisted of glycolipids and seaweed carotenoid, fucoxanthin. In the fucoxanthin-fed mice, WAT weight significantly decreased and UCP1 was clearly expressed in the WAT, while there was no difference in WAT weight and little expression of UCP1 in the glycolipids-fed mice. This result indicates that fucoxanthin upregulates the expression of UCP1 in WAT, which may contribute to reducing WAT weight.
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Keywords: Uncoupling protein 1; Uncoupling protein 2; White adipose tissue; Brown adipose tissue; Fucoxanthin; Antiobesity

A great deal of interest has been focused on adaptive thermogenesis by uncoupling protein (UCP) families (UCP1, UCP2, and UCP3) as a physiological defense against obesity, hyperlipidemia, and diabetes [1,2]. UCP are found in brown adipose tissue (BAT) (UCP1, UCP2, and UCP3), WAT (UCP2), skeletal muscle (UCP2 and UCP3), and brain (UCP4 and UCP5) [2,3]. UCP1 expression in BAT is known as a significant component of whole body energy expenditure and its dysfunction contributes to the development of obesity [4]. However, adult humans have very little BAT and most of fat is stored in white adipose tissue (WAT). Considered as breakthrough discoveries for an ideal therapy of obesity, regulation of UCP expression in tissues other than BAT by food constituent would be important.

Some dietary constituents have a strong influence on energy expenditure from a thermodynamic perspective. Polyunsaturated fatty acids, particularly those of n-3 family, had the ability to enhance thermogenesis [5].

☆ *Abbreviations:* UCP, uncoupling protein; BAT, brown adipose tissue; WAT, white adipose tissue; HPLC, high performance liquid chromatography; GC, gas chromatography; PPAR, peroxisome proliferator activated receptor.

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This ability of n-3 polyunsaturated fatty acids has been postulated to upregulate hepatic [6] and skeletal [7] UCP2 gene expression. Caffeine upregulated the mRNA expression of UCP1, 2, and 3 in BAT, and UCP2 and 3 in skeletal muscles, which would contribute to thermogenesis in obese mice [8]. Capsiate increased the levels of UCP1 protein and mRNA in BAT, UCP2 mRNA in WAT, and UCP3 mRNA in skeletal muscle in mice [9].

UCP2 and UCP3 are members of the mitochondrial anion carrier superfamily with high homology to UCP1, a well-characterized uncoupling protein playing a key role in facultative thermogenesis in rodents. Therefore, UCP2 and 3 have been expected to show a role in energy dissipation and a genetic linkage to obesity [2]. However, obesity was not induced in the UCP2 [10] and UCP3 [11,12] knockout mice. Thus, it is still unclear whether UCP2 and 3 also participate in thermoregulation [13]. They may have another physiological role [2,10,14]. Apart from UCP2 and UCP3, it is certain that UCP1 can potentially reduce excess abdominal fat. UCP1, usually expressed only in BAT, has been also found in WAT of mice overexpressing *Foxc2*, a winged helix gene, with a change in steady-state levels of several WAT and BAT derivative mRNAs [15]. This result suggests the possibility of UCP1 expression in WAT, which would be an increasingly attractive target for the development of antiobesity therapies. However, there has been no report on the dietary effect on both the gene and protein expressions of UCP1 in WAT. As the key molecular components become defined, screening for food constituent that increases energy dissipation is becoming a more attainable goal. This study showed the antiobesity effect of edible seaweed carotenoid, fucoxanthin, through protein and gene expressions of UCP1 in WAT.

Materials and methods

Sample preparation. Dried powder of seaweed (*Undaria pinnatifida*) after removing carbohydrate and protein was obtained from Riken Vitamin (Tokyo, Japan). The powder was extracted with chloroform/methanol (2:1, v/v). This powder contained 15%(w/w) lipids. Part of the lipid extract was used for the quantitative analysis of fucoxanthin and fucosterol by reversed phase high performance liquid chromatography (HPLC). HPLC experiments were carried out with a Hitachi HPLC-D7000 equipped with photodiode array detector. The column used was ODS UG-5 (250×4.6 mm i.d.). The mobile phase was methanol-acetonitrile (70:30, v/v). The flow rate was 1.0 ml/min. The detector was set at 450 and 210 nm for detecting fucoxanthin and fucosterol, respectively. Fucoxanthin (purity >98%) was prepared from *Undaria* lipids as described previously [16]. Standard fucoxanthin (purity >99.2%) was obtained by reversed phase HPLC purification. Fucosterol (purity >99%) was obtained from Extra Synthese. The quantitative analysis of both compounds was done with the calibration curve made using standard fucoxanthin and fucosterol. The lipids contained 9.6% fucoxanthin and 0.7% fucosterol. The lipid extract was also used for the analysis of different lipid class compositions. The lipid classes were separated by silica gel column chromatography by successive elution with chloroform, acetone/methanol (9:1, v/v), and methanol. Main lipid classes were glycolipids (68%) and triacylglycerol (21%). The total lipids were transmethylated using sodium methoxide (0.5 M) to obtain the fatty acid methyl esters. The methyl esters were further analyzed by gas chromatography (GC). Shimadzu GC-14B was used for fatty acid analysis. The GC was equipped with FID and Omegawax 320 fused silica capillary column ($30\ m \times 0.32$ mm i.d.). The detector, injector, and column temperatures were 260, 250, and 200 °C, respectively. The carrier gas was helium with a flow rate of 50 kPa. Main fatty acids of total lipids from *Undaria* were 18:4n-3 (25.3%), 20:5n-3 (14.2%), 16:0 (12.3%), 18:3n-3 (12.0%), 20:4n-6 (9.7%), 18:2n-6 (6.9%), and 18:1n-9 (5.2%).

Separation of fucoxanthin and glycolipids. *Undaria* lipids were washed with *n*-hexane. The residue was dissolved in methanol and cooled to 5 °C. Precipitation was used as fucoxanthin rich fraction, which consisted of 67.4% fucoxanthin. All of other components was glycolipids. The supernatant was separated by silicic acid column chromatography by successive elution with *n*-hexane/acetone (7:3, v/v), *n*-hexane/acetone (6:4, v/v), acetone, and methanol. Fractions eluted with acetone and methanol were combined and used as *Undaria* glycolipid fraction, which had little of other components.

Animal care. Male Wistar rats and female KK-Ay mice (5 and 3 weeks of age) were housed at 23 ± 1 °C and at 50% relative humidity with 12/12-h light/dark cycle. Animals had free access to drinking water and were fed a diet prepared according to the recommendations of American Institute of Nutrition (AIN-93G) [17]. After acclimation for 1 week by feeding the AIN diet, animals were randomly divided into each group of seven and given an experimental diet. The dietary fats for rats were 7% soybean oil (control), 6.5% soybean oil + 0.5% *Undaria* lipids, and 5% soybean oil + 2% *Undaria* lipids. Those for mice were 13% soybean oil (control), 12.5% soybean oil + 0.5% *Undaria* lipids, 11% soybean oil + 2% *Undaria* lipids, 12.6% soybean oil + 0.4% fucoxanthin rich fraction, and 11.2% soybean oil + 1.8% *Undaria* glycolipid fraction. After treatment with experimental diet for 4 weeks, starved animals were killed under ether anesthesia. Abdominal WAT and interscapular BAT (only for mice) were rapidly removed in their entirety, weighed, and frozen in liquid nitrogen for Western blot and RNA analyses. Abdominal WAT was composed of perirenal and epididymal adipose tissues for rats and perirenal, gonadal, retroperitoneal, and mesenteric adipose tissues for mice. The animal care and procedure were approved by the Animal Care and Use Committee of Hokkaido University.

Western blot analysis. BAT or WAT of mice (ca. 500 mg) was homogenized 5–10 volumes of a solution containing 10 mM Tris–HCl and 1 mM EDTA (pH 7.4) for 30 s with a Polytron. After centrifugation at 1500*g* for 5 min, the fat cake was discarded, and the infranant (fat-free extract) was used for Western blot analysis of UCP1 [18]. Total protein content in BAT and WAT was measured with a DC protein assay kit (Bio-Rad). The supernatants (BAT 5 mg protein/lane, WAT 20 mg protein/lane) were separated by 10% SDS–polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membrane. The membrane was incubated with an antibody against UCP1 (Sigma, Saint Louis, USA) for 1 h and then was incubated with a secondary antibody rabbit IgG-conjugated horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The membranes were treated with the reagents in the chemiluminescence detection kit (ECL system, Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. β-Actin was detected as a control with β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

RNA analysis. Mouse WAT (ca. 30 mg) was taken and stored in a RNA later storage solution (Sigma). Total RNA was separated using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Real time quantitative RT-PCR was done with an automated sequence detection system (Applied Biosystems). The PCR solution (50 μl) was composed of 25 μl Syber Green PCR Master Mix solution (Applied

Byosystems), 0.25 µl multiscribe reverse transcriptase (50 U/µl), 1.0 µl RNase inhibitor (20 U/µl), template RNA, each primer (200 nM), and RNA-free water. The primer sequences used for detection of UCP1, UCP2, and mouse glyceraldehyde-3-phosphate dehydrogenase (GADH; internal control) were as follows. Forward: 5′CTCAGGA TTGGCCTCTACGACTC3′ and reverse: 5′TTGGTGTACA TGGACATCGCA3′; for UCP1, forward: 5′CTGGTCGCCGGCC TGCAGCGC3′ and reverse: 5′GATCCCTTCCTCTCGTGCAAT3′ for UCP2, and forward: 5′GAAGGTCGGTGTGAACGGATT3′ and reverse: 5′GAAGACACCAGTAGACTCCACGACATA3′ for GADH [9]. PCR cycling conditions were 48 °C for 30 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, 60 °C for 1 min.

Statistical analysis. Data are expressed as means + SD. Statistical analyses between multiple groups were determined by ANOVA. Statistical comparisons were made by Scheffe's *F* test or Tukey's test. Analysis between two groups was determined using the unpaired Student *t* test. Differences with $P < 0.01$ or $P < 0.05$ were considered significant.

Results and discussion

We used lipids from *U. pinnatifida* (Wakame) for fucoxanthin source, which is one of the most popular edible seaweeds in Japan and Korea. Throughout the experimental period of 4 weeks, no significant differences were observed among the three groups of rats given the three different fat diets in either the mean body weight or the food intake. The weights of the liver, and other organs, other than WAT, were not different among the different groups. The weight of WAT, composed of perirenal and epididymal abdominal adipose tissues, was significantly lower in 2% *Undaria* lipid-fed rats than in the control group (Fig. 1A). In 0.5% *Undaria* lipid-fed rats, the weight of WAT was observed to be lower than in control, but not significantly. In the 2% *Undaria* lipid-fed obese mice (KK-Ay), the weight of WAT was also significantly lower than in the control group (Fig. 1B). Furthermore, body weight of mice fed 2% *Undaria* lipid was significantly ($P < 0.05$) lower than that of control, although there were no significant differences in the mean daily intake of diet between the both groups. The final average body weight was 34.0 ± 2.3 g for 2% *Undaria* lipid-fed mice, 41.0 ± 1.6 g for 0.5% *Undaria* lipid-fed mice, and 39.7 ± 1.3 g for control mice.

In order to confirm the active component of *Undaria* lipids, fucoxanthin rich fraction and *Undaria* glycolipid fraction were administered to obese KK-Ay mice. Fig. 2 shows dietary effects of both fractions on the WAT weight of obese mice. The WAT weight of fucoxanthin rich fraction-fed mice was significantly lower than that of control mice. However, there was no difference in the WAT weight of mice fed *Undaria* glycolipids and control diet. This result indicates that fucoxanthin is an active component for antiobesity effect of *Undaria* lipids.

Because of its capacity for uncoupled mitochondrial respiration, BAT has been implicated as an important site of facultative energy expenditure in small rodents. This has led to speculation that BAT normally functions to prevent obesity. In 2.0% *Undaria* lipid-fed mice, BAT weight was significantly greater than in control mice (Fig. 3A). However, there was no significant difference in UCP1 expression among the three different dietary groups (Figs. 3B and C). Thus, the decrease in abdominal fat pad weight found in *Undaria* lipid-fed mice could not be explained only by the energy expenditure in BAT mitochondria by UCP1. In contrast to rodents, humans have only minute amounts of BAT, and thereby the contribution of BAT to the regulation of energy balance has been claimed to be much less or negligible in humans. However, if the expression of UCP1, a key molecule

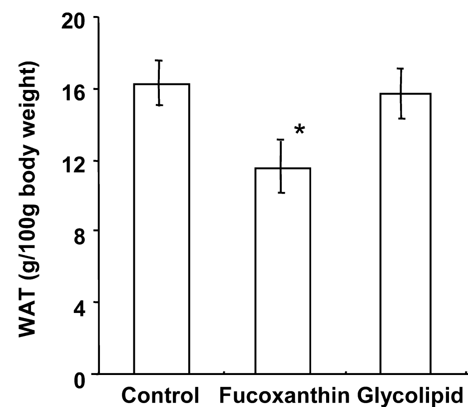

Fig. 2. Weight of WAT of mice fed fucoxanthin rich fraction, *Undaria* glycolipids fraction, and control diet. *Significantly different from control ($P < 0.01$).

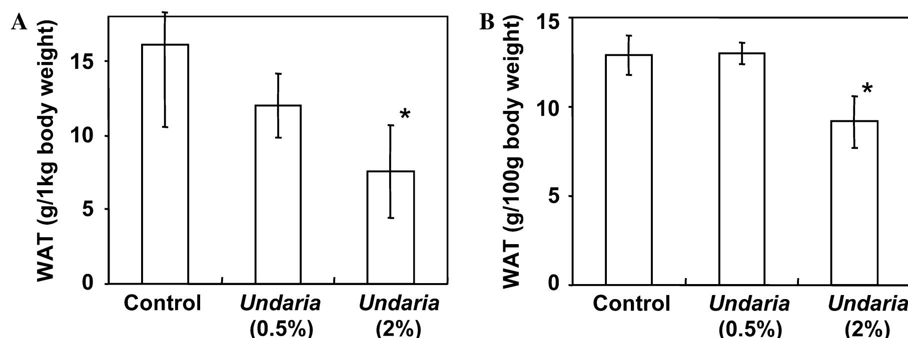

Fig. 1. Weight of WAT of rats (A) and mice (B) fed *Undaria* lipids and control diet. *Significantly different from control ($P < 0.01$).

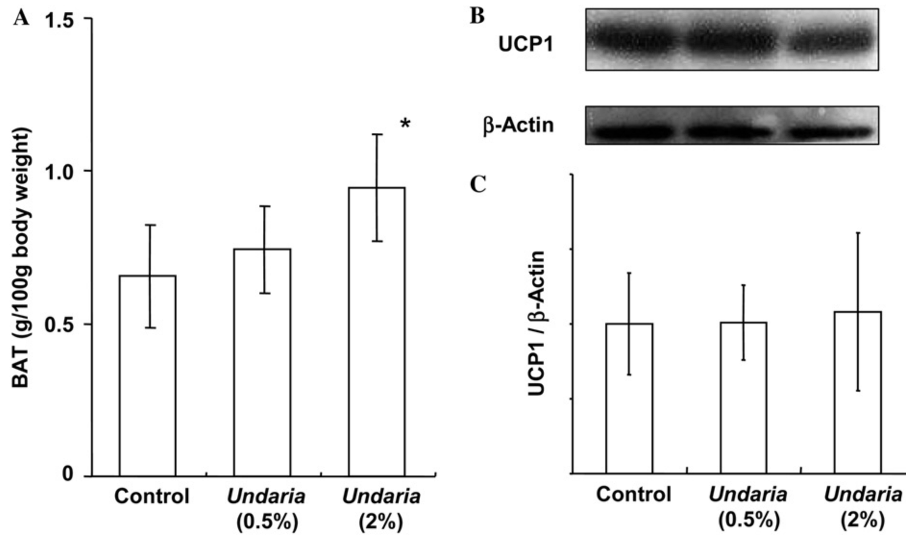

Fig. 3. Weight and UCP1 expression of BAT of mice fed *Undaria* lipids and control diet. (A) Weight of BAT. (B) Western blot analysis of UCP1. (C) UCP1 protein expression. *Significantly different from control ($P < 0.01$).

for BAT thermogenesis, can be activated in tissues other than BAT by food constituent, this would be good anti-obesity therapies for humans. As shown in Figs. 4A and B, UCP1 expression was found in WAT of *Undaria* lipid-fed mice, although there was little expression in that of control mice. Expression of UCP1 mRNA was also found in WAT of *Undaria* lipid-fed mice, but little expression in that of control (Fig. 4C). On the other hand, UCP2 expression in WAT decreased by feeding *Undaria* lipids as compared with control (Fig. 4D). These results show that decrease in WAT weight of *Undaria* lipid-fed mice would be due to the thermogenesis through UCP1 expression in WAT, but through UCP2 expression.

UCP1 expression in WAT was also found in fucoxanthin rich fraction-fed mice (Fig. 5), but little expression of UCP1 was found in WAT of mice fed *Undaria* glycolipids and control diets. This result confirmed the anti-obesity activity of seaweed carotenoid, fucoxanthin, by upregulation of UCP1 expression in WAT. Interest in UCPs increased with the discovery of proteins similar to UCP1, including UCP2 and UCP3. These proteins

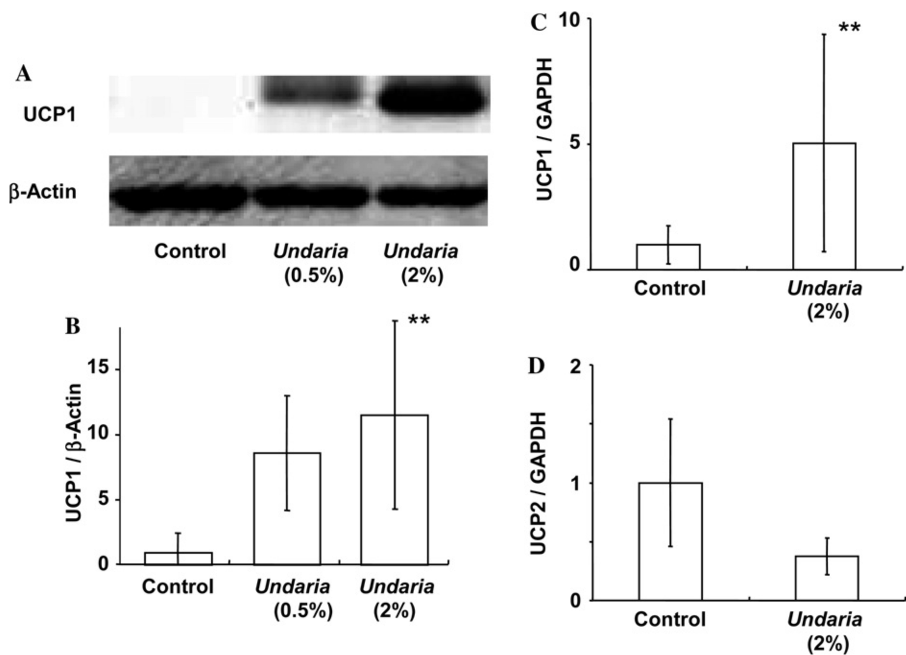

Fig. 4. UCP1 and UCP2 expressions in WAT of mice fed *Undaria* lipids and control diet. (A) Western blot analysis of UCP1. (B) UCP1 protein expression. (C) UCP1 mRNA expression. (D) UCP2 mRNA expression. **Significantly different from control ($P < 0.05$).

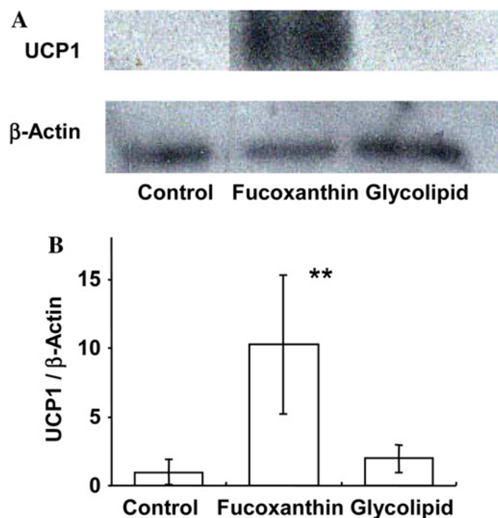

Fig. 5. UCP1 expression in WAT of mice fed fucoxanthin rich fraction and *Undaria* glycolipid fraction. (A) Western blot analysis of UCP1. (B) UCP1 protein expression. **Significantly different from control ($P < 0.05$).

are expressed in tissues besides BAT and, thus, are candidates to influence energy efficiency and expenditure. Since metabolic rate, metabolic efficiency, and obesity are integrated properties of the whole animal, researchers have produced mice lacking UCP2 [10] and UCP3 [11,12]. Surprisingly, despite UCP2 or UCP3, no consistent phenotypic abnormality was observed in the knockout mice. They were not obese and had normal thermogenesis. These results suggest that UCP2 and UCP3 are not a major determinant metabolic rate in normal condition but, rather, have other functions. Indeed, unexpected physiological or pathological implications of the UCP2 and UCP3 function were indicated [2], such as the possible UCP2 involvement in diabetes and in apoptosis. UCP3 have a diminished thermogenic response to the drug MDMA [14]. On the other hand, we found both protein and mRNA expressions of UCP1 in WAT of mice fed fucoxanthin, but UCP2 mRNA expression reduced, which will give a clue for new dietary antiobesity therapy. An enormous amount of data has been collected on thermogenesis in BAT through UCP1 expression. However, there has been no information on UCP1 expression in WAT induced by a diet. An excessive accumulation of fat in WAT induces some diseases such as type II diabetes. Direct heat production by fat oxidation in WAT, therefore, will reduce a risk of these diseases in humans.

An enhancer element of UCP1 gene promotes transcription that is both BAT-selective and responsive to β-adrenergic stimulation through camp [13]. This complex enhancer element has putative binding sites for the thyroid hormone receptor, retinoic acid receptor, and peroxisome proliferator-activated receptor-γ (PPARγ). The study of gene expression in BAT is of special interest because this has been regarded as the only tissue in the mammalian body that functions exclusively as a thermogenic organ. Treatment with β-carotene and α-carotene promoted UCP1 expression in BAT. This effect of carotenoids could be explained by their conversion to retinoic acid [18]. On the other hand, fucoxanthin has no potency as vitamin A. Furthermore, there was no significant difference in the UCP1 expression in BAT of *Undaria* lipid-fed mice as compared with that of control mice, although BAT of 2% *Undaria* lipid-fed mice was significantly greater than that of control. Because of a little amount of BAT in mice, it was difficult to explain the WAT weight decrease by UCP1 expression in BAT. Thus, a significant decrease in the WAT weight of rats and mice fed *Undaria* lipid diet (Fig. 1) would be due to UCP1 expression and energy dissipation via the generation of heat by UCP1 expression in the WAT. The present study showed that seaweed carotenoid, fucoxanthin, is an active component for the antiobesity effect of *Undaria* lipids.

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