Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model

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Received April 13, 2009; Accepted June 30, 2009

DOI: 10.3892/mmr_00000189

Abstract. Fucoxanthin, a characteristic carotenoid of brown algae, has been reported to exert an anti-diabetic effect in an obese murine model. Wakame (*Undaria pinnatifida*), an edible seaweed, is rich in fucoxanthin. This study examined the anti-obesity and anti-diabetic effects of fucoxanthin-rich wakame lipids (WLs) on high fat (HF) diet-induced obesity in mice. Mice were fed a high fat control (HF_C) or normal fat control (NF_C) diet for 10 weeks. The HF diet-fed group was administered a HF diet containing WLs for a further 5 weeks. Parameters related to diabetes and obesity conditions were evaluated and compared. The HF-WL diet, which was rich in fucoxanthin, significantly suppressed body weight and white adipose tissue (WAT) weight gain induced by the HF diet. Dietary administration of the HF diet resulted in hyperglycemia, hyperinsulinemia and hyperleptinemia in the mouse model. These perturbations were completely normalized in the HF-WL diet-fed group. Increased expression of monocyte chemoattractant protein-1 (MCP-1) mRNA expression was observed in HF_C mice, but was normalized in the HF-WL groups. Moreover, the HF-WL diet promoted mRNA expression of β3-adrenergic receptor (Adrb3) in WAT and glucose transporter 4 (GLUT4) mRNA in skeletal muscle tissues. These results suggest that dietary WLs may ameliorate alterations in lipid metabolism and insulin resistance induced by a HF diet. There is therefore a biochemical and nutritional basis for the application of fucoxanthin-rich WLs as a functional food to prevent obesity and diabetes-related disorders.

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Key words: anti-obesity, fucoxanthin, monocyte chemoattractant protein-1, glucose transporter 4, β3-adrenergic receptor, wakame lipids

Introduction

The modern-day tendency to consume nutritionally rich diets coupled with irrational dietary habits create physiological disorders. These lead to the accumulation of visceral fat, and finally result in obesity and related disorders, such as diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease (1,2). Long-term consumption of a high fat (HF) diet accelerates the development of obesity (3); hence, strategies to prevent obesity are of great importance. Fucoxanthin is a characteristic carotenoid of brown algae including edible species such as *Undaria pinnatifida* and *Hijikia fusiformis*. We recently reported the suppression of the development of white adipose tissue (WAT) by fucoxanthin in obese/diabetic KK-A^y mice (4,5). Expression of uncoupling protein 1 (UCP1), which plays an important role in energy expenditure, was induced by dietary fucoxanthin in WAT but not in brown adipose tissue; this UCP1 expression was responsible for the anti-obesity effect of fucoxanthin. Furthermore, dietary fucoxanthin regulated adipocytokine secretion, thus preventing hyperglycemia in a type 2 diabetes KK-A^y mouse model (5).

The adipocyte as an endocrine cell has recently been recognized for its role in the secretion of biologically active mediators, termed adipokines/chemokines, including leptin, adiponectin, resistin, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) (6-8). Some adipokines are reported to alter insulin sensitivity and glucose and lipid metabolism in muscle, liver and adipose tissues. The participation of macrophages in inflammatory responses by the release of pro-inflammatory mediators (TNF-α and MCP-1) under obese conditions has also been reported (6,9).

Skeletal muscle accounts for nearly 40% of body mass, and its role in the insulin-induced stimulation of glucose uptake (10,11) is well documented. Glucose transporter 4 (GLUT4) translocation is linked to reduced glucose utilization in insulin-resistant muscle, and a significant reduction in GLUT4 protein and mRNA levels in skeletal muscle has been reported in HF diet cases (12,13). In addition, transgenic animals with over-expressed or knocked out GLUT4 have provided significant insights into the role of GLUT4 in glucose homeostasis (14).

In the present study, we investigated the effect of wakame lipids (WLs) containing fucoxanthin on adipose tissue weight, and the role of fucoxanthin in attenuating hyperglycemia in mice with diet-induced obesity. Furthermore, the mechanism of fucoxanthin-modulated insulin resistance in adipose tissue and skeletal muscle was examined by determining glucose metabolism-related gene expression.

Materials and methods

Materials. Dried seaweed (*Undaria pinnatifida*) powder devoid of carbohydrates and protein as well as medium-chain triacylglycerols (MCTs) and vitamin E were obtained from Riken Vitamin (Tokyo, Japan). The main fatty acids of MCT were caprylic acid (C8:0; 61.4%) and capric acid (C10:0; 38.6%).

Fucoxanthin-rich wakame lipid preparation. WLs rich in fucoxanthin were prepared from dried seaweed powder by acetone extraction. The composition of the WLs was analyzed by high performance liquid chromatography (HPLC) as per previously published methods (15). The extracted WLs mainly consisted of glycolipids (68%), and had a fucoxanthin concentration of 10%. Reversed-phase HPLC was carried out using the Hitachi L-7000 system on a Develosil ODS-UG-5 (250x4.6 mm i.d., 5.0 μm particle size; Nomura Chemical Co.) fitted with a guard column (10x4.0 mm i.d.) containing the identical stationary phase. A mixture of methanol and acetonitrile (70:30, v/v) at a flow rate of 1.0 ml/min was used as the mobile phase. Fucoxanthin was monitored at 450 nm using a UV-Vis detector, and fucoxanthin identification was accomplished by comparison with the standard preparation as previously described (16).

Dietary studies using model mice. All the animal experiment protocols had the prior approval of the Ethics Committee of Hokkaido University for experimental animal care. Male C57BL/6J mice (8 weeks of age) were purchased from Japan Crea Co. (Tokyo, Japan). The mice were housed at 23±1˚C and at 50% humidity under a 12/12-h light/dark cycle. Animals were acclimatized for one week, with *ad libitum* access to drinking water and a control (AIN-93G) diet containing normal fat (NF) levels, consisting of 7% fat (soybean oil), 20% casein, 39.7486% cornstarch, 10% sucrose, 13.2% dextrinized cornstarch, 5% cellulose, 3.5% AIN-93G mineral mix, 1% AIN-93G vitamin mix, 0.3% L-cystine, 0.25% coholine bitartrate and 0.0014% *Tert*-butylhydroquinone. Post-acclimatization, the mice were randomly divided into 5 groups (n=6 each). Four of the groups were fed a HF diet for 10 weeks. The fifth group was fed a NF diet, referred to as the negative control (NF_C). The HF diet consisted (w/w) of 30% fat (20.7% lard, 7% soybean oil, 2.2% medium-chain triglyceride, 0.1% vitamin E), 25.8% casein, 18.1284% cornstarch, 10% sucrose, 6.0202% dextrinized cornstarch, 5% cellulose, 3.5% AIN-93G mineral mix, 1% AIN-93G vitamin mix, 0.3% L-cystine, 0.25% coholine bitartrate and 0.0014% *Tert*-butylhydroquinone.

After 10 weeks of being fed the HF or NF diet, the NF_C group continued to receive the NF diet for another 5 weeks. Of the four HF diet-fed groups, for the next 5 weeks one received the NF diet (HF-NF_C), while the remaining three groups received a HF diet (HF_C), WLs with 1.06% fucoxanthin (HF-WL1) or WLs with 2.22% fucoxanthin (HF-WL2). The composition (g/kg diet) of the diets is shown in Table IA. The fatty acid composition of each diet was analyzed by gas chromatography, as previously described (4).

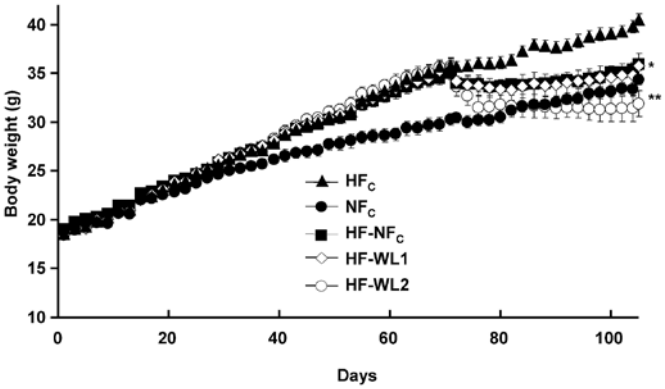

Figure 1. Effect of body weight on C57BL/6J mice fed the experimental diets. Values are presented as the mean ± SE (n=6). *P<0.05 and **P<0.01 vs. HF_C.

At the end of experimental period, the groups were starved for 12 h before being sacrificed under anesthesia. All organs including WAT and muscle were rapidly removed, weighed and stored in RNA later™ Storage Solution (Sigma Chemical Co., St. Louis, MO, USA). Blood was collected immediately in heparin-coated capillary tubes (BD Vacutainer® Plus Plastic Tubes; Nippon Becton Dickison Co., Ltd., Tokyo, Japan) and stored at -80±1˚C until further analysis.

mRNA analysis. Total RNA was extracted from the uterine WAT using the RNeasy® Lipid Tissue Mini Kit and from muscle using the RNeasy Fibrou Mini Kit (both from Qiagen, Tokyo, Japan) according to the manufacturer's protocol. Then, cDNA was synthesized from total RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems Japan Ltd., Tokyo, Japan). Real-time quantitative RT-PCR analysis was performed with an automated sequence detection system (ABI PRISM 7500; Applied Biosystems Japan). PCR cycling conditions were 40 cycles at 95˚C for 15 sec and 60˚C for 1 min. mRNA expression of TNF-α, MCP-1, liptin, UCP2, β3-adrenergic receptor (Adrb3), GLUT4 (Slc2a4), peroxisome proliferator-activated receptor δ (PPARδ), UCP3, carnitine palmitoyltransferase 1a (CPT1α) and GAPDH was measured using the Taqman® Gene Expression Assays (Applied Biosystems Japan). PCR primers (TNF-α, Mm00445641_m1; MCP-1, Mm99999056_m1; leptin, Mm00434759_m1; UCP2, Mm00495907_g1; Adrb3, Mm00442669_m1; GLUT4, Mm00436615_m1; PPARδ, Mm00803186_g1; UCP3, Mm00494074_m1; CPT1α, Mm00550438_m1; GAPDH, Mm99999915_g1) were purchased from Applied Biosystems Japan. Each PCR reaction was normalized to GAPDH.

Blood glucose, insulin and leptin concentrations, and lipid parameters of plasma. Blood glucose levels were determined using a blood glucose level monitor (Glutest Neo; Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan) after 34 days of administration of the experimental diet. Other parameters were measured on the final day of the experimental period.

Table I. Experimental diets.

A, Composition of the experimental diets.

Ingredients[a]	HF_C	NF_C HF-NF_C	HF-WL1	HF-WL2
Lard[b]	207	-	196.4	184.8
Soybean oil[c]	70	70	70	70
Medium-chain triglyceride[d]	22	-	22	22
Vitamin E[d]	1	-	1	1
Wakame lipids[d]	-	-	10.6	22.2
Corn starch[e]	181.284	397.486	181.284	181.284
Casein[e]	258	200	258	258
Dextrinized cornstarch[e]	60.202	132	60.202	60.202
Sucrose[f]	100	100	100	100
AIN-93 mineral mixture[e]	35	35	35	35
AIN93 vitamin mixture[e]	10	10	10	10
L-cystine[g]	3	3	3	3
Choline bitartrate[g]	2.5	2.5	2.5	2.5
Cellulose[e]	50	50	50	50
Tert-Butylhydroquinone[g]	0.014	0.014	0.014	0.014
Total	1000	1000	1000	1000

B, Fatty acid composition of the experimental diets.

Fatty acid (wt %)	HF_C	NF_C HF-NF_C	HF-WL1[h]	HF-WL2[i]
8:0	3.7	ND	3.6	3.7
10:0	2.8	ND	2.4	2.6
16:0	20.8	10.9	20.2	20.3
18:0	11.1	3.9	10.9	10.4
18:n-9	33.6	23.0	33.1	32.4
18:n-7	2.3	2.2	2.2	1.8
18:2n-6	18.3	51.8	18.3	18.5
18:3n-3	1.5	5.7	1.8	2.1

[a]Measured in g/kg diet. [b]Showa Chemical Co., Ltd. (Tokyo); [c]Wako Pure Chemical Industries (Osaka); [d]Riken Vitamin Co. (Tokyo); [e]Clea Japan (Tokyo); [f]Kanto Chemical Co., Inc. (Tokyo, Japan); [g]Sigma-Aldrich (St. Louis, MO, USA). [h]Same as HF_C diet with the addition of 1.06% wakame lipids; [i]same as HF_C diet with the addition of 2.22% wakame lipids. ND, not detected.

Insulin and leptin levels in the blood plasma were analyzed by commercial ELISA kits (Mouse Insulin ELISA kit, U-type; Shibayagi, Gunma, Japan; and Mouse Leptin Assay Kit (L)-IBL; IBL Co., Ltd., Gunma, Japan). Total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, phospholipid and free fatty acid concentrations were determined using respective test kits at the Hakodate Ishikai Kennsa Center.

Statistical analysis. The means were analyzed by analysis of variance (ANOVA). In case of significance, mean separation was accomplished by the unpaired Student's t-test using Excel Tokei software (Esumi Co., Ltd., Tokyo, Japan).

Results

Changes in body and adipose tissue weight. Changes in the body weight of the experimental animals over the course of the study are presented in Fig. 1. In the first 10 weeks, the body weights of mice in the HF diet groups (HF_C, HF-NF_C, HF-WL1 and HF-WL2) were ~5 g higher than those of the NF_C group, indicating the induction of obesity due to a HF diet. During the additional 5-week period, the HF_C group continued to gain body weight, while weight gain in the WL diet-fed groups (HF-WL1 and 2) was significantly suppressed ($P<0.01$, $P<0.05$). The final body weight of the HF-WL1 group

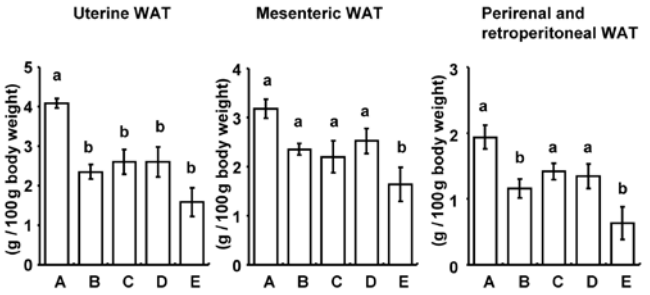

Figure 2. Relative adipose tissue weight of C57BL/6J mice. (A) HF_C, (B) NF_C, (C) HF-NF_C, (D) HF-WL1 and (E) HF-WL2. Values are presented as the mean ± SE (n=6). Differences were considered significant at P<0.05.

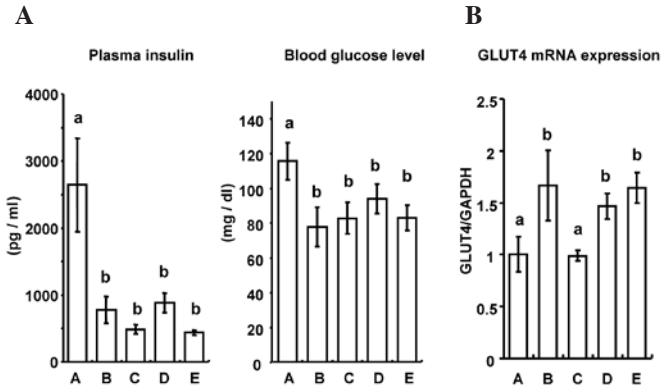

Figure 3. (A) Plasma insulin and blood glucose levels in C57BL/6J mice. (B) Glucose transporter 4 (GLUT4) mRNA expression in the muscle tissue of C57BL/6J mice. (A) HF_C, (B) NF_C, (C) HF-NF_C, (D) HF-WL1 and (E) HF-WL2. Values are presented as the mean ± SE (n=6). Differences were considered significant at P<0.05.

was 35.8±3.1 g, and was at the same level as in the NF_C group. There was no significant difference (P>0.05) in food intake (g/mouse) among the HF groups (HF_C, 75.9±4.6; HF-WL1, 72.7±5.3; HF-WL2, 73.1±13.3) throughout the experimental period.

WAT weight gain was suppressed in the groups fed a HF-WL diet. This suppression was dose dependent (Fig. 2). The WAT weight of the HF-WL2 group was significantly (P<0.05) lower than that of HF_C group. Fatty acid composition did not differ between the HF-WL1 and HF-WL2 groups (Table IB).

Plasma parameters. Plasma HDL cholesterol, triacylglycerol, phospholipid and free fatty acid concentrations did not differ among the experimental groups (Table II). Total cholesterol was higher in the HF diet groups (HF_C, HF-WL1 and HF-WL2) (P<0.05) than in the NF diet groups (NF_C and HF-NF_C). On the other hand, LDL cholesterol levels were decreased in the HF-WL1 and 2 groups compared to the HF_C group.

Plasma leptin content was significantly (P<0.05) lower in HF-WL1 and 2 groups compared to the HF_C group (Table II). Plasma insulin and blood glucose levels were higher in the HF_C group than in the NF_C group (Fig. 3A). However, the plasma insulin levels of the HF-WL1 and 2 groups were the same as those of the NF groups (NF_C and HF-NF_C).

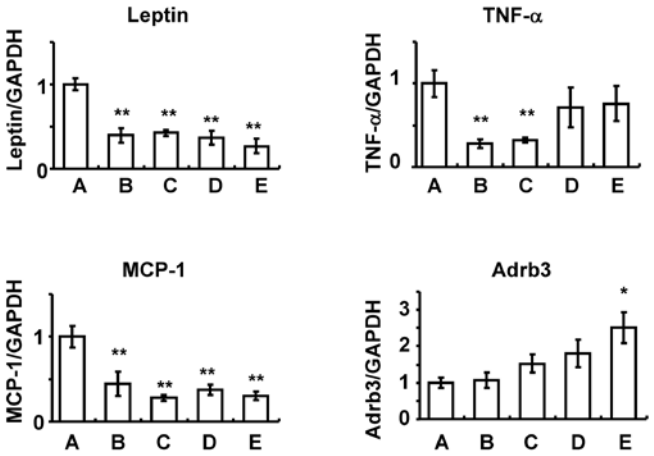

Figure 4. Leptin, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and β3-adrenergic receptor (Adrb3) mRNA expression in the WAT of C57BL/6J mice. (A) HF_C, (B) NF_C, (C) HF-NF_C, (D) HF-WL1 and (E) HF-WL2. Values are presented as the mean ± SE (n=6). *P<0.05 and **P<0.01 vs. HF_C.

Gene expression related to glucose and lipid metabolism in white adipose tissue. mRNA expression related to glucose and lipid homeostasis in WAT was measured by real-time quantitative RT-PCR (Fig. 4). Leptin mRNA expression was significantly (P<0.01) suppressed in the HF-WL groups compared to the HF_C group, and was correlated with the level of plasma leptin (Table II). TNF-α mRNA levels in WAT in the NF groups (NF_C and HF-NF_C) were markedly decreased, but were lower in the HF-WL groups than in the HF_C group. MCP-1 mRNA expression was improved in the HF-WL groups compared to the HF_C group. Adrb 3 mRNA levels were higher in the HF-WL groups compared to the HF_C group.

Gene expression related to glucose and lipid metabolism in muscle. GLUT4 mRNA levels in skeletal muscle tissue were markedly lower in the HF_C group compared to the NF_C group (Fig. 3B). However, GLUT4 mRNA levels in the HF-WL groups were restored to levels observed in the NF_C group, despite the intial administration of a HF diet. Other mRNA expression levels (PPARδ UCP2, UCP3, CPT1α) related to fatty acid oxidation in muscle tissue did not change in any of the groups (data not shown).

Discussion

In this study, wakame lipids were used as a source of fucoxanthin. Wakame is the most popular edible seaweed in Japan and Korea, and contains various polysaccalides and proteins. These water soluble components have immune modulation and anti-hypertention activity (17), and are used as industrial food materials. Residue after the extraction of these components contains abundant WLs, and can thus be used as a resource for functional food material.

WLs restored blood glucose and insulin levels, and suppressed MCP-1 mRNA expression in WAT. A high fat diet induces hyperinsulinemia and hyperglycemia (3). MCP-1 is released in adipose tissue, induces macrophage accumulation

Table II. Plasma lipid parameters and leptin levels of C57BL/6J mice fed the experimental diets.

	HF_C	NF_C	HF-NF_C	HF-WL1	HF-WL2
Total cholesterol (mg/dl)	168±5[a]	127±8[b]	123±4[b]	192±10[a]	185±7[a]
HDL cholesterol (mg/dl)	65±2	67±5	61±3	71±3	66±2
LDL cholesterol (mg/dl)	13±1.8[a]	7±0.4[b]	7±0.4[b]	9±0.9[a,b]	7±0.4[b]
Triacylglycerol (mg/dl)	56±9	84±15	72±13	82±19	62±20
Phospholipid (mg/dl)	278±12	251±15	239±8	289±6	266±12
FFA (μEq/l)	1424±116	1807±199	1686±187	1599±67	1346±167
Leptin (ng/dl)	13.0±2.8[a]	7.6±2.4[a]	6.0±0.7[b]	3.2±2.1[b]	1.5±1.0[b]

HDL, high density lipoprotein; LDL, low density lipoprotein; FFA, free fatty acids. Values are presented as the mean ±SE (n=6). Differences were considered significant at $P<0.05$.

and stimulates macrophages to produce pro-inflammatory mediators (11). This suggests that a WL diet improves insulin resistance, suppressing inflammatory reactions in WAT. Our previous study revealed that fucoxanthin and fucoxanthinol (a metabolized component of fucoxanthin) affect peroxisome proliferator-activated receptor γ (PPARγ) and promote gene expression related to lipid metabolism in adipocytes (4,15). Hence, it can be presumed that WLs relieve inflammation in adipose tissue, thus having an anti-diabetic effect.

The administration of a WL diet additionally promoted the recovery of blood glucose uptake to muscle cells by regulating GLUT4 mRNA expression. Although the HF-NF_C group recovered from hyperglycemia due to the later administration of a normal fat diet, GLUT4 mRNA levels did not recover compared to the WL diet groups. Glucose homeostasis may therefore not have been normalized in the HF-NF_C group. Some thiazolidinedione family drugs increase GLUT4 mRNA expression in the muscle tissue of type 2 diabetes mellitus (18,19), similar to the effect of WLs rich in fucoxanthin.

In a previous study, we demonstrated that fucoxanthin, the active component in WLs, has an anti-obesity effect (5). The main mechanism of this effect was shown to be the induction of UCP1 in WAT by fucoxanthin. In this study, in the HF-WL diet groups, increases in body and WAT weight were suppressed compared to the HF_C group. Leptin suppresses appetite and controls body weight (20). However, obese individuals have leptin resistance due to high leptin levels in the blood. Hence, plasma leptin levels can be used as an index of body fat accumulation. Low leptin levels detected in the groups fed a HF-WL diet indicated the anti-obesity effect of WLs in mice with HF diet-induced obese conditions.

The fatty acid composition of the HF-WL diet did not differ from that of the HF_C diet. We suggest that fucoxanthin was responsible for promoting energy expenditure, as evidenced by lower WAT weights in the HF-WL groups. However, UCP1 protein and mRNA expression were not detected in WAT in this study (data not shown). Similar observations were reported in a β3 adrenergic agonist experiment with C57BL/6J mice (21), and were attributed to the low response of these mice to UCP1 enhancement stimulation. It is possible that WLs rich in fucoxanthin promote energy expenditure by different means.

Recently, to examine the effect of fucoxanthin on WAT, we analyzed changes in gene expression using DNA microarrays in a type 2 diabetes model using KK-A^y mice (unpublished data). Adrb3 mRNA expression was increased in the WAT of mice fed a fucoxanthin diet. Adrb3 is mainly expressed in brown adipose tissue and WAT, and is considered to be responsible for lipolysis and thermogenesis (22,23). The degree of obesity is correlated with the extent of loss of Adrb3 gene expression in WAT (24). The results of the current study suggest that WLs may promote sensitivity to sympathetic nerve stimulation and the up-regulation of fat oxidation in WAT.

The present study demonstrated that dietary WLs rich in fucoxanthin reduced body fat accumulation in a mouse model with diet-induced obesity conditions. WLs were additionally shown to modulate blood glucose and insulin levels, possibly by suppressing MCP-1 and promoting Adrb3 and GLUT4 expression. These findings indicate the functionality of WLs as an anti-obesity and anti-diabetic functional food.

Acknowledgements

This study was supported by the 'Research and Development Program for New Bio-industry Initiatives' of the Bio-oriented Technology Research Advancement Institution, and by a grant-in-aid (#18 · 54022) from the Japan Society for the Promotion of Science (JSPS). Research was also supported by a fellowship from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Lew EA and Garfinkel L: Variations in mortality by weight among 750,000 men and women. J Chronic Dis 32: 563-576, 1979.
2. Olefsky JM, Revers RR, Prince M, Henry RR, Garvey WT, Scarlett JA and Kolterman OG: Insulin resistance in non-insulin dependent (type II) and insulin dependent (type I) diabetes mellitus. Adv Exp Med Biol 189: 176-205, 1985.
3. Buettner R, Schölmerich J and Bollheimer LC: High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity 15: 798-808, 2007.
4. Maeda H, Hosokawa M, Sashima T, Funayama K and Miyashita K: Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem Biophys Res Commun 332: 392-397, 2005.
5. Maeda H, Hosokawa M, Sashima T and Miyashita K: Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-A^y mice. J Agric Food Chem 55: 7701-7706, 2007.

6. Hotamisligil GS, Shargill NS and Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87-91, 1993.
7. Matsuzawa Y, Shimomura I, Kihara S and Funahashi T: Importance of adipocytokines in obesity-related diseases. Horm Res 60: 56-59, 2003.
8. Shoelson SE, Lee J and Goldfine AB: Inflammation and insulin resistance. J Clin Invest 116: 1793-17801, 2006.
9. Hotamisligil GS, Arner P, Caro JF, Atkinson RL and Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95: 2409-2415, 1995.
10. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI and Kahn BB: Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 409: 672-673, 2001.
11. Wellen KE and Hotamisligil GS: Inflammation, stress, and diabetes. J Clin Invest 115: 1111-1119, 2005.
12. Kahn BB and Pedersen O: Suppression of GLUT4 expression in skeletal muscle of rats that are obese from high fat feeding but not from high carbohydrate feeding or genetic obesity. Endocrinology 132: 13-22, 1993.
13. Kim Y, Tamura T, Iwashita S, Tokuyama K and Suzuki M: Effect of high-fat diet on gene expression of GLUT4 and insulin receptor in soleus muscle. Biochem Biophys Res Commun 202: 519-526, 1994.
14. Charron MJ, Katz EB and Olson AL: GLUT4 gene regulation and manipulation. J Biol Chem 274: 3253-3256, 1999.
15. Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T and Miyashita K: Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. Int J Mol Med 18: 147-152, 2006.
16. Kotake-Nara E, Kushiro M, Zhang H, Sugawara T, Miyashita K and Nagao A: Carotenoids affect proliferation of human prostate cancer cells. J Nutr 131: 3303-3306, 2001.
17. Murata M, Sano Y, Ishihara K and Uchida M: Dietary fish oil and *Undaria pinnatifida* (wakame) synergistically decrease rat serum and liver triacylglycerol. J Nutr 132: 742-747, 1985.
18. Armoni M, Harel C and Karnieli E: Transcriptional regulation of the GLUT4 gene: from PPAR-gamma and FOXO1 to FFA and inflammation. Trends Endocrinol Metab 18: 100-107, 2007.
19. Olefsky JM and Saltiel AR: PPAR gamma and the treatment of insulin resistance. Trends Endocrinol Metab 11: 362-368, 2000.
20. Matsuzawa Y: The metabolic syndrome and adipocytokines. FEBS Lett 580: 2917-2921, 2006.
21. Watson PM, Commins SP, Beiler RJ, Hatcher HC and Gettys TW: Differential regulation of leptin expression and function in A/J vs. C57BL/6J mice during diet-induced obesity. Am J Physiol Endocrinol Metab 279: E356-E365, 2000.
22. Krief S, Lönnqvist F, Raimbault S, Baude B, van Spronsen A, Arner P, Strosberg AD, Ricquier D and Emorine LJ: Tissue distribution of beta 3-adrenergic receptor mRNA in man. J Clin Invest 91: 344-349, 1993.
23. Lönnqvist F, Thöme A, Nilsell K, Hoffstedt J and Arner P: A pathogenic role of visceral fat beta 3-adrenoceptors in obesity. J Clin Invest 95: 1109-1116, 1995.
24. Collins S, Daniel KW and Rohlfs EM: Depressed expression of adipocyte beta-adrenergic receptors is a common feature of congenital and diet-induced obesity in rodents. Int J Obes Relat Metab Disord 23: 669-677, 1999.