

## Anti-Obesity Activity of Alkali-Treated Fucoxanthin in C57BL/6 J Mice Fed a High-Fat Diet

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### Summary

Fucoxanthin (Fx) is a marine xanthophyll primarily found in brown algae, known for its anti-obesity, anti-tumor, and antioxidant properties. A previous study showed that alkali treatment alters the chemical structure of fucoxanthin, resulting in two main compounds: isofucoxanthinol (Iso FxOH) and fucoxanthinol hemiketal (FxOH HK). However, the physiological activities of alkali-treated Fx remain unclear. Here, for the first time, we investigated the physiological effects of dietary IsoFxOH and FxOH HK on obesity in mice, compared to Fx. Six-week-old male C57BL/6 J mice were fed a high-fat diet (HFD) as the HFD-cont group, an HFD containing 0.05% (w/w) of Fx, Iso FxOH, or FxOH HK as the experimental groups, or AIN-93G as the control group for 8 weeks. There were no significant differences in final body weight or white adipose tissue weight between the groups. However, in mesenteric white adipose tissue, the expression level of *Lpl* was suppressed in mice fed HFD containing 0.05% (w/w) FxOH HK. Conversely, the expression of *Srebf1*, which is involved in *de novo* lipogenesis, was upregulated by HFD supplemented with 0.05% (w/w) FxOH HK. Additionally, FxOH HK upregulated the expression of *Cpt1a*, which is involved in fatty acid oxidation. These results suggest that the products of alkali-treated Fx may alleviate obesity in mice fed a high-fat diet, primarily by reducing fatty acid uptake in adipose tissue.

### Introduction

Obesity, primarily resulting from excessive triglyceride (TG) accumulation in adipose and non-adipose tissues, is a global public health issue<sup>1</sup>. Obesity has two main consequences: hypertrophy of adipose tissue and the secretion of pathogenic cytokines<sup>2</sup>. These pathogenic cytokines can lead to various diseases, such as type 2 diabetes<sup>3,4</sup>, hypertension<sup>5,6</sup>, atherosclerosis<sup>7</sup>, cancer<sup>8,9</sup>, and others. Adipose tissue is the primary energy storage organ derived from dietary intake. There are two main pathways for lipid accumulation in adipose tissue: lipid uptake from lipid-rich lipoproteins and synthesis from non-lipid precursors, a process known as *de novo* lipogenesis (DNL)<sup>10</sup>. DNL primarily occurs in the liver and adipose tissues. When lipids are excessively synthesized in the liver, they are transported into the circulatory system by very low-density lipoproteins (VLDL) and hydrolyzed in the capillaries of muscle or adipose tissues<sup>11</sup>. During this process, lipoprotein lipase (LPL) is a critical enzyme involved in the up-

take of circulating triglycerides by muscle or adipose tissues as it degrades triglyceride-rich lipoproteins<sup>12</sup>. Therefore, reducing fat intake or synthesis and increasing fat consumption are two common therapeutic strategies for preventing obesity.

Marine algae are a potential source of bioactive compounds, which have attracted significant attention in recent years<sup>13,14</sup>. Fucoxanthin (Fx) is one of the main marine carotenoids extracted from brown algae, such as *Undaria* (wakame in Japanese) and *Laminaria* (konbu in Japanese), and has recently been reported to possess numerous biological activities<sup>15</sup>. Kotake-Nara et al. revealed that Fx induces apoptosis in PC-3 human prostate cancer cells through caspase-3 activation<sup>16</sup>. Sugawara et al. reported that Fx possesses anti-angiogenic activity, which might slow cancer progression of cancer<sup>17</sup>. Maeda et al. found that Fx exhibited anti-obesity activity by improving metabolic thermogenesis<sup>18</sup>. Liu et al. indicated that Fx exerts antioxidant effects by activating the Nrf2/ARE pathway<sup>19</sup>. Based on this evidence, Fx may be a promising

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candidate for functional food and drug development owing to its potential pharmacological activity against many life-style-related diseases.

Carotenoids are known to be unstable when exposed to high temperatures, light, and changes in surrounding pH. Additionally, Yusof et al. reported that fucoxanthin undergoes changes in color and antioxidant activity when exposed to heat, low pH, or light<sup>20</sup>. Due to fucoxanthin's instability under varying environmental conditions, it is essential to clarify how its biological activity changes once its chemical structure is altered. A previous study showed that Fx changes its chemical structure after alkali treatment, resulting in two main products: isofucoxanthinol (Iso FxOH) and fucoxanthinol hemiketal (FxOH HK) (Fig. 1 A)<sup>21</sup>. However, the biological activity of Iso FxOH and FxOH HK remains unknown. Here, for the first time, we investigated the physiological activities of these two compounds through dietary administration in relation to obesity in C57BL/6 J mice fed a high-fat diet (HFD) and compared their effects with those of Fx.

## Materials and methods

### *Alkali treatment of Fx for HPLC analysis*

Two mg of Fx powder (ALNUR Co., Ltd, Tokyo, Japan) was dissolved in 500  $\mu$ L of diethyl ether. After mixing with 500  $\mu$ L of 5% KOH-methanol solution, the mixture was incubated in the dark for 1–48 hours at room temperature. Subsequently, 2 mL of chloroform and 900  $\mu$ L of water were added to the mixture, which was then vortexed and centrifuged at 1700 g for 10 minutes at 4 °C. The upper layer was removed, and 1 mL of methanol and 900  $\mu$ L of water were added. The mixture was centrifuged again under the same conditions as described above. This process was repeated five times and, the resulting lower layer was collected. After drying by nitrogen blowing, the residue was re-dissolved in methanol and stored at –80°C until use.

### *HPLC condition*

HPLC analysis was performed using a Shimadzu series instrument (Shimadzu, Japan) equipped with a photodiode array (PDA) detector (SPD-M20A, Shimadzu, Japan). Signals were transmitted to the LC Solution software for analysis and peak area calculation at wavelengths of 450 nm for Fx and Iso FxOH, and 420 nm for FxOH HK. Chromatographic separation was achieved using a TSKgel ODS-80 Ts (4.6  $\times$  250 mm, 5  $\mu$ m) column. The HPLC was performed using a gradient mobile phase system consisting of acetonitrile: methanol: water (75:15:10, by vol.) con-

taining 0.1% ammonium acetate (phase A), and ethyl acetate/methanol (7:3, v/v) containing 0.1% ammonium acetate (phase B) at a flow rate of 1 mL/min. The pump was programmed as follows: Phase B was increased from 0% to 100% over 20 min, maintained at 100% for 15 min, decreased back to 0% over 5 min, and maintained at 0% for 5 min (total gradient time: 45 min). A 10  $\mu$ L sample was injected into the system, and the column temperature was kept at 40°C.

### *Preparation of carotenoids for animal diet*

Fx, Iso FxOH, and FxOH HK used in animal experiments were kindly provided by ALNUR Co., Ltd. (Tokyo, Japan). Iso FxOH and FxOH HK were separated from alkali-treated Fx. Briefly, Fx (2 g) was dissolved in ethanol (200 mL), and Dowex 1 (Anion Exchange Resin, OH form) was added as an alkaline reactant. The mixture was then stirred for 22 h at room temperature. After stirring, the mixture was filtered and the residue was dissolved in acetone (10 mL). The solution was separated using Isolera One (a column containing 200 g of SiO<sub>2</sub>) and eluted with hexane/acetone and 10% ethanol/acetone. Finally, 801.7 mg Iso FxOH and 422.9 mg FxOH HK were prepared.

### *Animals and diets*

Six-week-old male C57BL/6JmsSlc mice (6 weeks of age) were purchased from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan). All mice were housed individually and maintained on a 12-hour light/dark cycle at a temperature of 23  $\pm$  1°C. After a 1-week acclimatization period on the AIN-93G diet (D10012GM; Research Diets, NJ, USA), the mice were randomly divided into five groups (n = 8 per group) and had ad libitum access to drinking water. During the 8-week period, mice in each group were fed either a high-fat diet or a low-fat diet. The mice fed the AIN-93G diet as low-fat diet were named as the control group, while those fed the high-fat diet (HFD; D12451M, Research Diets, NJ, USA) without any additional experimental compounds were named as the HFD-cont group. The mice fed the HFD containing 0.05% Fx, Iso FxOH, or FxOH HK were named as the HFD-Fx group, HFD-Iso FxOH group, and HFD-FxOH HK group, respectively. Dietary components of each group are listed in Table 1 and energy composition of each diet are listed in Table 2. Body weight and food intake were monitored throughout the study period. After 8 weeks of feeding each experimental food, the mice were food-deprived for 15 h and euthanized under isoflurane anesthesia. Blood and organs were rapidly collected, weighed, and immediately frozen in liquid nitrogen. The white adipose tissue (WAT), liver,

**Table 1** Compositions of experimental diet

	Control	HFD-cont	HFD-Fx	HFD-Iso FxOH	HFD-FxOH HK
	g				
Milk casein	20	22.807	22.807	22.807	22.807
L-Cystine	0.3	0.342	0.342	0.342	0.342
Corn starch	39.7486	8.301	8.301	8.301	8.301
Dextrinized starch	13.2				
Maltodextrin 10		11.403	11.403	11.403	11.403
Sucrose	10	19.704	19.704	19.704	19.704
Cellulose	5	5.701	5.701	5.701	5.701
Soybean oil	7	5.004	5.004	5.004	5.004
Lard		20.24	20.24	20.24	20.24
Mineral mix	3.5	1.14	1.14	1.14	1.14
DiCalcium phosphate		1.482	1.482	1.482	1.482
Calcium carbonate		0.627	0.627	0.627	0.627
Potassium itrate, 1 H2O		1.881	1.881	1.881	1.881
Vitamin mix	1	1.14	1.14	1.14	1.14
Choline bitartrate	0.25	0.228	0.228	0.228	0.228
Tert-butylhydroquinone	0.0014				
Fucoxanthin			0.05		
Isofucoxanthinol				0.05	
Fucoxanthinol hemiketal					0.05
Total	100	100	100	100	100

**Table 2** Energy compositions of experimental diet

	AIN-93G diet		HFD	
	g%	kcal%	g%	kcal%
Protein	20	20	24	20
Carbohydrate	64	64	41	35
Fat	7	16	24	45
Vitamin	0.01		0.0114	
Mineral	0.035		0.0114	
Total		100		100
kcal/g	4.0		4.73	

and skeletal muscle were partly stored in RNA Later™ solution (Ambion, CA, USA) at  $-80^{\circ}\text{C}$  until used for quantitative real-time PCR analyses. All animal experimental protocols were approved by the Animal Experimentation Committee of Kyoto University for the care and use of experimental animals (Approval number R5-26).

### Biochemical analysis

Plasma was prepared by centrifugation at  $400 \times g$  for 15 min at  $4^{\circ}\text{C}$  and stored at  $-80^{\circ}\text{C}$  until use. Plasma con-

centrations of total glycerides (TG), free cholesterol, total cholesterol (TC), HDL cholesterol, non-esterified fatty acids (NEFA), and glucose were measured using commercially available kits (TG E, F-Cho E, T-Cho E, HDL-C E, NEFA, and Glu C II, respectively; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Plasma aspartate aminotransferase and alanine aminotransferase concentrations were measured using a commercial kit (GOT GPT C II).

### RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from mesenteric white adipose tissue, liver, and skeletal muscle using Sepasol reagent (Nacalai Tesque) according to the manufacturer's instructions. The isolated RNA was reverse transcribed into cDNA using ReverTra Ace® qPCR RT Master Mix with gDNA remover (Toyobo, Osaka, Japan). For qRT-PCR, the cDNA was diluted and mixed with iQ SYBR Green Supermix (Bio-Rad Laboratories) containing  $1 \mu\text{mol/L}$  PCR primer. Real-time qRT-PCR was performed using a DNA Engine Option system (Bio-Rad Laboratories).

The PCR conditions were as follows: 95°C for 3 minutes (initial denaturation), followed by 40 cycles of 95°C for 15 seconds (denaturation) and 60°C for 30 seconds (extension). Primer sequences are listed in Table 3. The expression level of each gene was normalized using  $\beta$ -actin as an internal control.

### Statistical analysis

Data were expressed as the mean  $\pm$  SE for the animal experiments. Data were compared using a one-way analysis of variance followed by Dunnett's test or Tukey's test. Differences were considered statistically significant at  $p < 0.05$ .

## Results and discussion

### Time-dependent changes in the chemical structure of alkali-treated Fx

To confirm the time-dependent changes in the chemical structure of Fx during alkali treatment, the treated Fx was analyzed using HPLC-PDA (Fig. 1B). The peak corresponding to Fx was detected at a retention time of 7 min. After 1 hour of alkali treatment, the Fx peak disappeared entirely, with new peaks emerging at 4.25 minutes (Iso FxOH) and 6.25 minutes (FxOH HK). The peak area of Iso FxOH gradually decreased, whereas that of FxOH HK increased over time, which was consistent with previous

findings<sup>21)</sup> (Fig. 1 C).

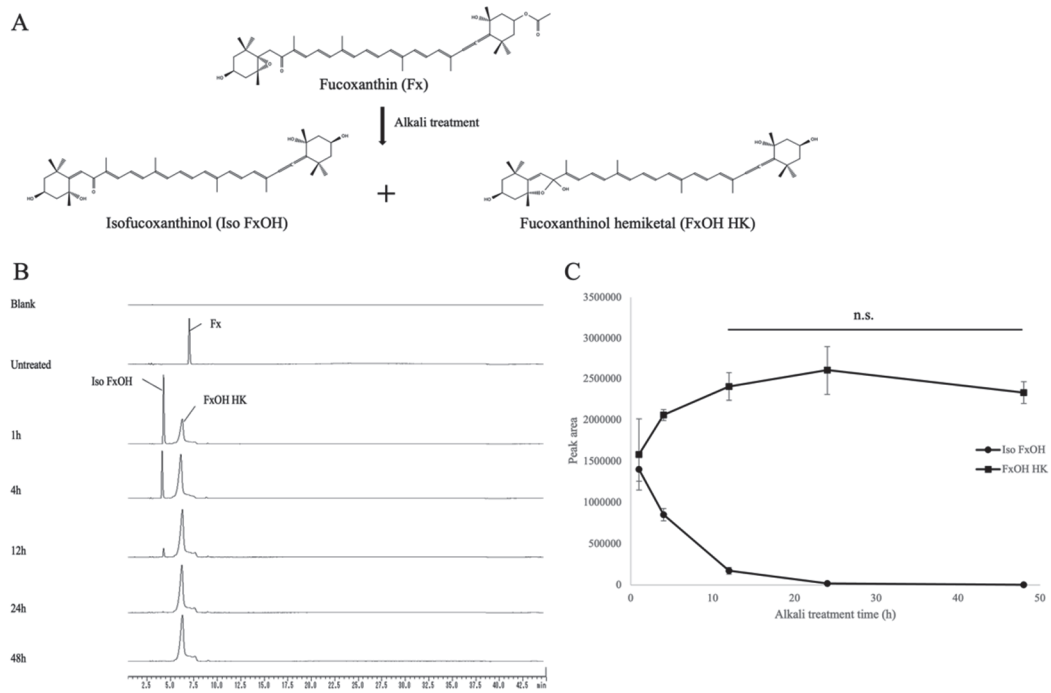
### Effect of dietary Fx, Iso FxOH or FxOH HK on biological parameters in mice

To investigate the effects of the oral administration of Fx, Iso FxOH, and FxOH HK in mice, body weight, food intake, liver weight, and various adipose tissues were measured (Fig. 2). After 8 weeks of experimental period, there was a trend of increased body weight gain in the control group compared to that in the HFD-cont group ( $p = 0.053$ ). Compared with the HFD-cont group, mice fed a HFD containing Iso FxOH or FxOH HK exhibited a tendency toward weight reduction, although this was not statistically significant (Fig. 2B). In terms of tissue weight, the HFD containing Iso FxOH or FxOH HK also tended to suppress weight gain, particularly in mesenteric white adipose tissue (mWAT) and epididymal white adipose tissue (eWAT) (Fig. 2D). However, mice in HFD-Fx group showed a slight increase in body weight and adipose tissue weight compared to the HFD-cont group (Fig. 2B and D).

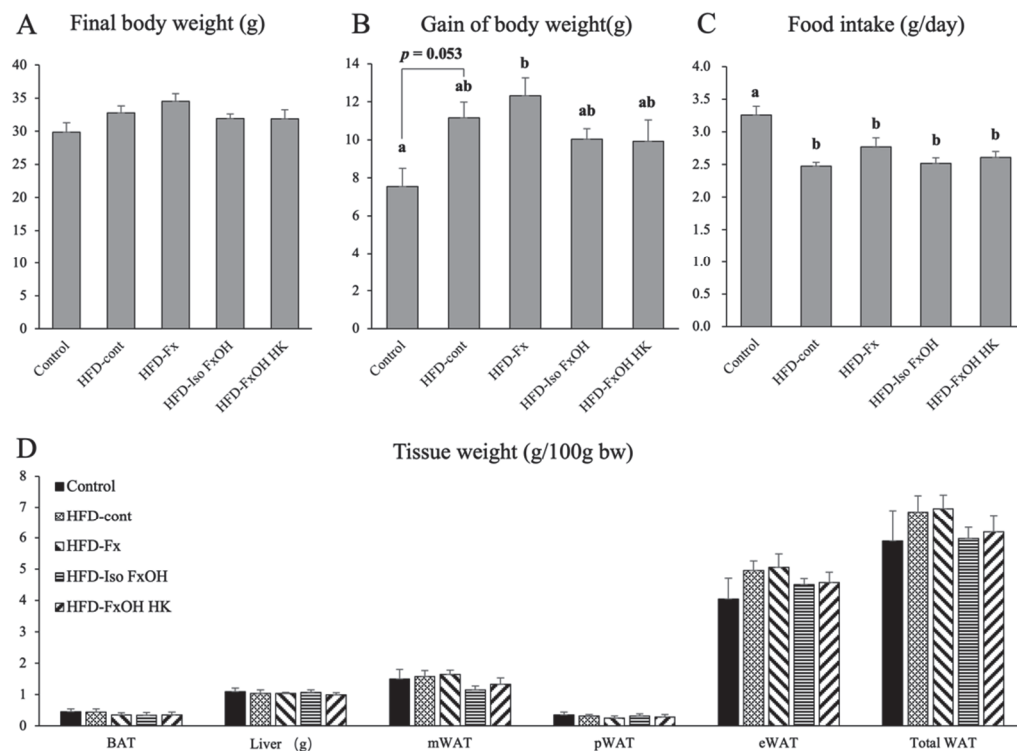
Plasma parameters related to lipid metabolism are summarized in Table 4. There were no significant differences in the plasma concentrations of non-esterified fatty acids (NEFAs), glucose, triglycerides (TG), HDL cholesterol, AST, or ALT among the groups. However, mice in HFD-Fx group or HFD-Iso FxOH group showed a significant increase in plasma free cholesterol and total cholesterol

**Table 3** Primers of quantitative real time RT-PCR

Gene name	Forward (from 5' to 3')	Reverse (from 3' to 5')
<i><math>\beta</math>-actin</i>	CCTCTATGCCAACACAGTGC	GTACTTGCCTCAGGAGGAG
<i>Acc</i>	AAACTGCAGGTATCCCAACTCTTC	CTGTGGAACATTTAAGATACGTTTTCGAAAA
<i>Acox1</i>	ACCTTCACTTGGGCATGTTC	TTCCAAGCCTCGAAGATGAG
<i>Cpt1a</i>	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
<i>Fabp4</i>	GTGAAAACCTTCGATGATTACATGAA	GCCTGCCACTTTCCTTGTG
<i>Fas</i>	CCTGGAACGAGAACACGATCT	AGACGTGTCACTCCTGGACTTG
<i>G6pd</i>	GGTACCTACAAGTGGGTGAA	AGATGGTGAAAAGGGAAGAT
<i>Lpl</i>	AGCCCCAGTCGCCTTCTCTCT	TGCTTTGCTGGGGTTTTCTTCAATCA
<i>Me1</i>	AGGGCACATTGCTTCAGTTC	TGTACAGGGCCAGTTTACCC
<i>Nr1h3</i>	TGCCATCAGCATCTTCTCTG	GGCTCACCAGCTTCATTAGC
<i>Pgc1a</i>	GAAGTGGTGTAGCGACCAATC	AATGAGGGCAATCCGTCTTCA
<i>Pgc1b</i>	TCCTGTAAAAGCCCGGAGTAT	GCTCTGGTAGGGCAGTGA
<i>Ppara</i>	TACTGCCGTTTTTACAAGTGC	AGGTCGTGTTTACAGGTAAGA
<i>Pparg</i>	GCCCTTTGGTGACTTTATGG	GGCGGTCTCCACTGAGAATA
<i>Scd1</i>	ACAGTCCAGGGCCAACAGT	GGCACCTTACACAGCCAGTT
<i>Srebf1</i>	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGCCCAG
<i>Ucp3</i>	CCTACGACATCATCAAGGAGAAGTT	TCCAAAGGCAGAGACAAAGTGA



**Fig. 1** Chemical structures of fucoxanthin (Fx), isofucoxanthinol (Iso FxOH) and fucoxanthinol hemiketal (FxOH HK) (A); HPLC chromatograms of Fx, Iso FxOH, and FxOH HK at 450 nm after different time of alkali treatment (B); Peak areas of Iso FxOH at 450 nm and FxOH HK at 420 nm during alkali treatment (C). Values are means  $\pm$  standard deviation ( $n = 8$ ). Significance was determined by Dunnett's test,  $p < 0.05$ .



**Fig. 2** Effect of Fx, Iso FxOH, or FxOH HK on body weight (A), gain of body weight (B), food intake (C), and tissue weight (D) after oral administration for 8 weeks. Values represent means  $\pm$  standard error ( $n = 8$ ). Significance was determined by Turkey's test, bars with different letters are considered significantly different from each other ( $p < 0.05$ ). BAT, brown adipose tissue; mWAT, mesenteric adipose tissue; pWAT, perirenal adipose tissue; eWAT, epididymal adipose tissue.

**Table 4** Effect of Fx, Iso FxOH and FxOH HK on plasma parameters in C57BL/6 J mice.

	Control	HFD-cont	HFD-Fx	HFD-Iso FxOH	HFD-FxOH HK
NEFAs (mEq/L)	1.18 ± 0.21	0.77 ± 0.08	0.78 ± 0.05	0.94 ± 0.07	0.81 ± 0.1
Free cholesterol (mg/dL)	34.42 ± 0.83 <sup>a</sup>	31.74 ± 1.52 <sup>a</sup>	44.08 ± 2.34 <sup>b</sup>	47.91 ± 1.53 <sup>b</sup>	32.74 ± 1.04 <sup>a</sup>
Glucose (mg/dL)	161.69 ± 10.31	151.97 ± 8.44	185.56 ± 9.75	167.73 ± 10.24	177.86 ± 13.95
TG (mg/dL)	124.98 ± 12.44	114.05 ± 6.83	127.90 ± 8.54	122.13 ± 4.81	111.33 ± 4.51
TC (mg/dL)	168.20 ± 4.96 <sup>a</sup>	167.43 ± 6.15 <sup>a</sup>	198.73 ± 10.85 <sup>b</sup>	205.02 ± 4.34 <sup>b</sup>	169.04 ± 5.72 <sup>a</sup>
HDL-C (mg/dL)	105.46 ± 5.88	102.51 ± 8.39	105.67 ± 7.29	117.52 ± 1.76	98.21 ± 2.93
AST (Karmen)	32.77 ± 1.19	35.65 ± 4.54	42.62 ± 4.19	34.17 ± 5.51	35.94 ± 2.88
ALT (Karmen)	12.53 ± 0.59	15.76 ± 2.62	15.03 ± 1.14	18.01 ± 3.4	22.73 ± 4.62

Values represent the means ± standard error (n = 8). Significance was determined using Tukey's test; bars with different letters were considered significantly different ( $p < 0.05$ ). NEFAs, non-esterified fatty acid; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

levels compared to the HFD-cont group (Table 4). This change can be explained by the findings of Beppu et al., who reported that dietary Fx downregulates the hepatic levels of LDLR and SR-B1 proteins, which are involved in cholesterol uptake in the liver, leading to an increase in cholesterol concentration in the plasma<sup>22</sup>. Considering that fucoxanthinol is the primary metabolite of Fx in vivo, we hypothesized that dietary Iso FxOH operates via the same mechanism<sup>23</sup>.

#### **Effect of dietary Fx, Iso FxOH or FxOH HK on gene expressions in mWAT, liver, and skeletal muscle**

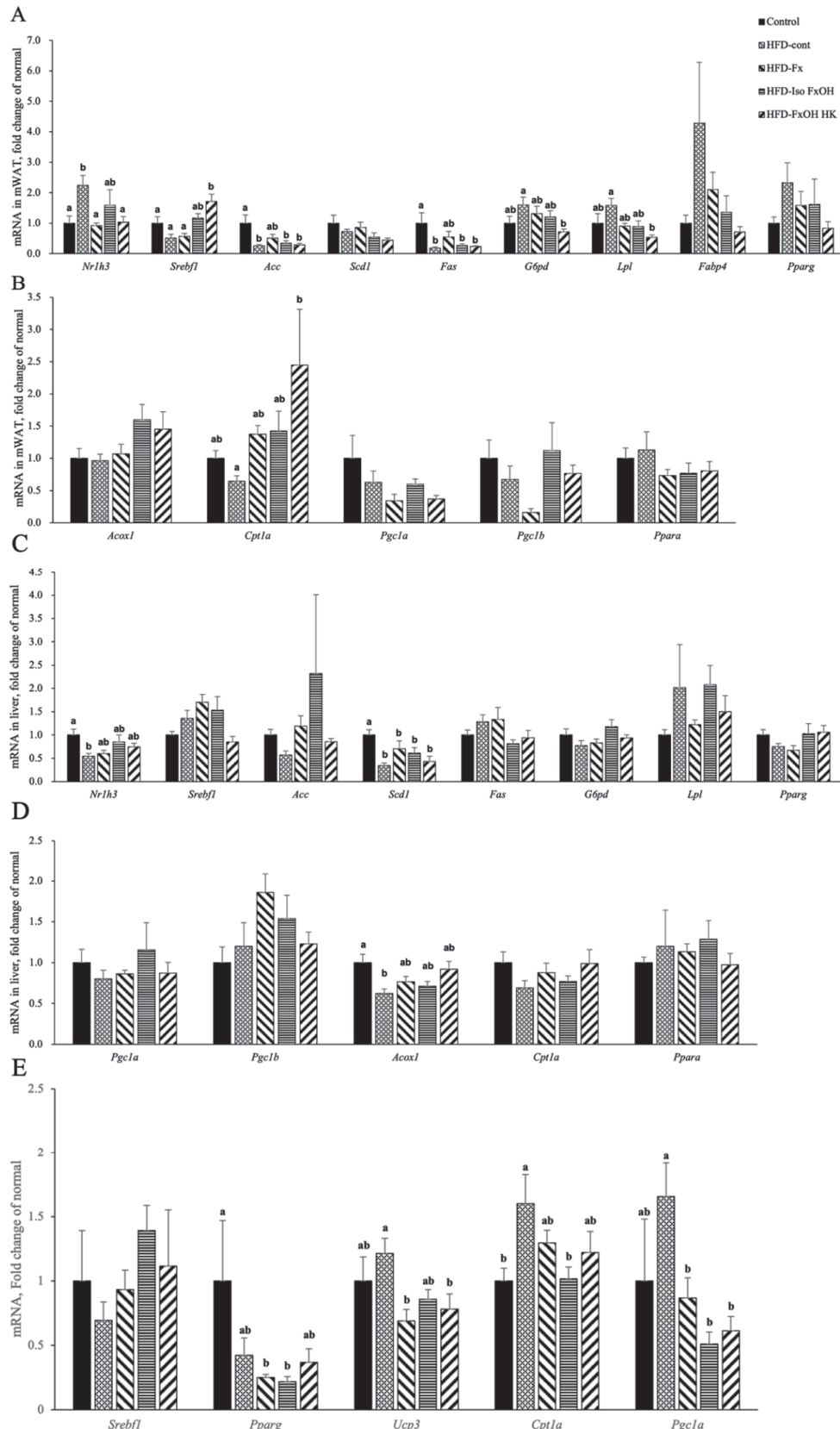
In mWAT, the expression of *Nr1h3*, which encodes the transcription factor liver X receptor  $\alpha$  (LXR $\alpha$ ), was significantly upregulated in the HFD-cont group compared to the control group. However, this upregulation was attenuated by all three experimental diets, with significant downregulation observed in HFD-Fx and HFD-FxOH HK groups (Fig. 3 A). The expression of *Srebf1*, a critical transcription factor involved in DNL, was slightly downregulated in the HFD-cont group compared to that in the control group but was significantly restored in the HFD-FxOH HK group. Additionally, the expression of *Acc* and *Fas* was significantly downregulated in the HFD-cont group compared to that in the control group. However, there were no significant differences in the expression of *Scd1*, *Fas*, or *Acc*, which are regulated by *Srebf1*, among HFD mice (Fig. 3 A). Moreover, dietary FxOH HK significantly attenuated the gene expression levels of *G6pd* and *Lpl* (Fig. 3 A) and significantly increased the gene expression level of *Cpt1a* which is involved in fatty acid oxidation (Fig. 3B), compared to the HFD-cont group. In the liver, however, there were no significant differences in the expression levels of any genes involved in lipid metabolism among HFD-fed mice (Fig. 3 C and D). In skeletal muscle,

dietary Fx, Iso FxOH, or FxOH HK significantly reversed the changes in the expression of genes involved in fatty acid oxidation, including *Ucp3*, *Cpt1a*, and *Pgc1a*, which were affected by HFD (Fig. 3E).

Liver X receptors (LXRs) are members of the nuclear receptor superfamily that play a regulatory role in lipid metabolism<sup>24</sup>. LXRs also regulate the sterol regulatory element-binding protein-1c (SREBP-1c). However, our results indicated that *Srebf1* was upregulated, while *Nr1h3* was downregulated in mWAT in the HFD-FxOH HK group compared to the HFD-cont group. LPL is a critical enzyme involved in the uptake of circulating triglycerides by the muscle or adipose tissue. In our study, dietary FxOH HK significantly decreased the expression level of *Lpl* in mWAT, suggesting a reduction in fatty acid uptake into white adipose tissue. Therefore, we speculate that the reduced entry of fatty acids into the adipose tissue may have feedback effects on the activation of DNL, including the regulation of *Srebf1* levels in the adipose tissue. Bartelt et al. found an increased expression of DNL-related genes, including *Fasn*, *Scd1*, and *Elovl6*, in adipocyte-specific LPL-knockout mice<sup>25</sup>. Moreover, *G6pd* is involved in NADPH synthesis, which is essential for fatty acid synthesis and adipose tissue inflammatory<sup>26</sup>. Downregulated expression of *G6pd* in the mWAT was observed in the HFD-FxOH HK group compared to that in the HFD-cont group.

ACOX and CPT1 $\alpha$  are key enzymes involved in fatty acid oxidation, while the PGC1 family is crucial coactivator that regulate mitochondrial biogenesis in adipose tissue. In this study, we found a significant upregulation of *Cpt1a* expression in mWAT with dietary FxOH HK, and an upward trend in *Cpt1a* expression with dietary Fx or Iso FxOH. Muscles are the primary fat-consuming organs that utilize fatty acids to produce ATP. Here, we observed significantly restored gene expression levels of *Ucp3*,





**Fig. 3** Effect of Fx, Iso FxOH or FxOH HK on various gene expression levels of genes involved in lipid metabolism after oral administration for 8 weeks. The gene expression levels of lipogenesis markers (A) or FA oxidation markers (B) in mWAT. The gene expression level of lipogenesis markers (C) or FA oxidation markers (D) in liver. The gene expression levels of lipogenesis markers or FA oxidation markers in skeletal muscle (E). The values represent means  $\pm$  standard error ( $n = 8$ ). Significance was determined by Turkey's test, bars with different letters are considered significantly different from each other ( $p < 0.05$ ). mWAT, mesenteric adipose tissue; *Acc*, acetyl-CoA carboxylase; *Acox1*, acyl-CoA oxidase 1; *Cpt1a*, carnitine palmitoyltransferase 1 $\alpha$ ; *Fabp4*, fatty acid binding protein 4; *Fas*, fatty acid synthase; *G6pd*, glucose-6-phosphate dehydrogenase; *Lpl*, lipoprotein lipase; *Pgc1a*, peroxisome proliferative activated receptor  $\gamma$  coactivator 1 $\alpha$ ; *Pgc1b*, peroxisome proliferative activated receptor  $\gamma$  coactivator 1 $\beta$ ; *Ppara*, Peroxisome proliferator-activated receptor  $\alpha$ ; *Pparg*, Peroxisome proliferator-activated receptor  $\gamma$ ; *Scd1*, stearyl CoA desaturase 1; *Srebf1*, sterol regulatory element binding protein 1; *Ucp3*, uncoupling protein 3.

*Cpt1a* and *Pgc1a* in skeletal muscle after 8 weeks in HFD-Fx, HFD-Iso FxOH, or HFD-FxOH HK groups compared to HFD-cont group.

### Summary

To our knowledge, this study is the first to examine the dietary effects of the two main products of alkali-treated Fx on HFD-induced obesity. In the present study, there were no significant differences in body weight gain or adipose tissue weight between the control and HFD-cont groups. Based on our results, the AIN-93G diet used in this study may not be an appropriate choice for a normal diet because of its high carbohydrate content<sup>27)</sup> (Table 2). Although various studies have reported that Fx has anti-obesity activity, we did not observe a reduction in body weight or adipose tissue weight in C57BL/6 J mice after eight weeks of feeding a diet containing 0.05% Fx, suggesting that a low dose of Fx does not affect HFD-induced obesity in C57BL/6 J mice. Maeda et al. reported that Fx significantly decreased the abdominal WAT weight in KK-Ay mice at a dose of 0.2%, which was four times higher than the dose used in our study<sup>28)</sup>. Kim et al. also reported that low doses of Fx (0.015%/0.03%) did not prevent obesity in C57BL/6 J mice fed a high-fat/high-sucrose/high-cholesterol (HFC) diet or a high-fat/high-sucrose (HFS) diet<sup>29)</sup>. However, in this study, a decreasing trend in body weight and adipose tissue weight was observed in mice fed the HFD containing 0.05% Iso FxOH or FxOH HK, suggesting that Iso FxOH and FxOH HK may have stronger anti-obesity activities than Fx at lower doses. In conclusion, although further experimental data are required, Iso FxOH and FxOH HK show promise as anti-obesity functional substances. Future experiments could be improved by reevaluating the normal diet and extending the duration of the animal experiments.

### Conflict of interests

ALNUR Co. Ltd. provided financial support for the author T.S. M. T. and Y. Y. are employees of ALNUR Co., Ltd. The other authors declare no conflicts of interest.

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