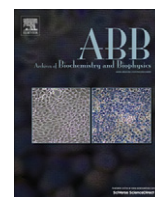




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# Combination of fucoxanthin and conjugated linoleic acid attenuates body weight gain and improves lipid metabolism in high-fat diet-induced obese rats

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## ABSTRACT

The present study investigated the effects of combined fucoxanthin (Fc) and conjugated linoleic acid (CLA) on high-fat diet-induced obese rats. Thirty five rats were divided into four groups, fed a high-fat diet (Control, 15% fat, wt/wt), supplemented with low Fc (FCL, 0.083 mg/kg/bw), high Fc (FCH, 0.167 mg/kg/bw) and FCL (0.083 mg/kg/bw) plus CLA (0.15 g/kg/bw) (FCL + CLA) for 52 d. Body weight and white adipose tissue (WAT) weight were significantly suppressed in FCL + CLA group than those in control group. WAT weight was also markedly attenuated in FCL and FCH groups. Accumulation of hepatic lipid droplets and the perirenal adipocyte size of FCL, FCH and FCL + CLA groups were diminished compared to control group. Serum total cholesterol level in FCH group, triacylglycerol and leptin levels in FCL, FCH and FCL + CLA groups, and glucose concentration in FCH and FCL + CLA groups were significantly decreased than those in control group. The mRNA expression of adiponectin, adipose triacylglycerol lipase, carnitine palmitoyltransferase 1A was remarkably up-regulated in FCL, FCH and FCL + CLA groups. These results suggest that Fc and FCL + CLA could reduce serum levels of triacylglycerol, glucose and leptin, and FCL + CLA could exert anti-obesity effects by regulating mRNA expression of enzymes related to lipid metabolism in WAT of diet-induced obesity rats.

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## Introduction

Obesity, recognized as a major public health problem worldwide, is closely related to many chronic diseases in both humans and animals such as diabetes mellitus, cardiovascular disease, digestive disease, respiratory disease and various cancers [1–3]. The excessive fat accumulation observed in obesity leads to the dysregulation of adipocytokine production in white adipose tissue (WAT). Adipose tissue, the energy reserve organ, plays an important role in regulating energy metabolism in organisms [4]. Adipocyte dysfunction is strongly associated with the development of obesity. It is accepted that specific regulation of gene expression in adipocytes is one of the most important targets for the intervention of

obesity. In addition, leptin and adiponectin, which is known to play an important role in maintaining insulin sensitivity and glucose homeostasis, is reduced in obese rats.

Fucoxanthin (Fc), an edible seaweed carotenoid that is characterized by a unique structure including an allenic bond and 5,6-monoepoxide, differs from that of common carotenoids such as  $\beta$ -carotene and lycopene [5]. Fc is mainly present in marine plants such as *Undaria pinnatifida*, *Sargassum fulvellum*, *Laminaria japonica* and *Hizikia fusiformis* [6]. mRNA of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1) is overexpressed in WAT of diabetic/obese KK-A<sup>y</sup> mice [7], the latter of which also induces the over-production of inflammatory adipocytokines [8,9] and inhibits insulin-dependent glucose uptake, thus leading to insulin resistance [10]. TNF- $\alpha$  and IL-6 are important pro-inflammatory adipocytokines that influence insulin sensitivity [11,12]. In addition, the expression of adiponectin, one of adipocytokines, correlates with insulin sensitivity. Remarkably, insulin resistance was completely reversed by a combination of physiological doses of adiponectin and leptin [13]. Therefore, the suppressive effects of Fc on the development of obesity and diabetes may depend on changes in the production of adipocytokines in WAT.

More recently, a crude mixture of conjugated linoleic acid (CLA) isomers has been shown to reduce body fat and enhance fat-free

**Abbreviations:** ATGL, adipose triacylglycerol lipase; BAT, brown adipose tissue; CLA, conjugated linoleic acid; CPT1A, carnitine palmitoyltransferase 1A; DG, diacylglycerol; FA, fatty acid; Fc, fucoxanthin; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; PPAR- $\gamma$ , peroxisome proliferator-activated receptor gamma; TC, total cholesterol; TG, triacylglycerol; TNF- $\alpha$ , tumor necrosis factor-alpha; UCP, uncoupling protein; WAT, white adipose tissue.

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mass in animals and humans [14,15]. In addition, the treatment of CLA during adipocyte differentiation reduces lipid accumulation and inhibits the expression of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), which is a nuclear receptor that activates genes involved in lipid storage and metabolism [16,17]. Of the two major isomers of CLA (10, 12 and 9, 11 isomers), the 10, 12 isomer is specifically responsible for the antiobesity effects [18–21]. The potential mechanisms of CLA on weight loss include the regulation of energy metabolism, adipogenesis, inflammation, lipid metabolism and apoptosis [22].

In the present study, we investigated the effects of Fc and FCL + CLA on body weight and adipose tissue weight, serum lipid profile, and obesity-related parameters in serum/plasma and gene expressions of lipid-regulating enzymes in perirenal WAT of diet-induced obesity rats.

## Materials and methods

### Materials

CLA was purchased from Cognis Chemicals Co., Ltd. China. The Fc oil, which contains 1% Fc, 81.3% modified starch, 17.2% seaweed crude extract, and 0.5% natural vitamin E, was obtained from Beijing Gingko Group Biological Technology Co., Ltd., China.

### Animals and diets

Thirty-five male Sprague Dawley (SD) rats, aged 3 week old, were obtained from Zhejiang University Laboratory Animal Center (Hangzhou, China). The rats were housed at  $23 \pm 1^\circ\text{C}$  and at 50% humidity with a 12 h light/12 h dark cycle. After acclimation for 1 week by feeding pellets of commercial chow, rats were randomly divided into four groups, fed a high-fat diet containing approximately 15% fat (wt/wt, Control,  $n = 5$ ), a high-fat diet plus 0.083 mg/kg/bw Fc (FCL,  $n = 10$ ), a high-fat diet plus 0.167 mg/kg/bw Fc (FCH,  $n = 10$ ) or a high-fat diet plus 0.083 mg/kg/bw Fc and 0.15 g/kg/bw CLA (FCL + CLA,  $n = 10$ ). The formula of the high-fat diet comprises 79% GB/T 14924.9 diet (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, AQSIQ, 2001), 10% lard, 10% yolk powder and 1% cholesterol. The rats had free access to food and water *ad libitum*. Food intake and body weight were measured daily and twice a week, respectively.

After feeding the control and experimental diets for 45 days, rats were starved for 12 h and sacrificed with decapitation. Blood was collected into EDTA-treated vacuum tubes. Plasma was obtained from blood samples after centrifugation at 3000 rpm for 10 min at  $4^\circ\text{C}$ . After collecting the blood, liver, white fat tissue from four regions (epididymal, perirenal, mesenteric and inguinal), and scapular brown fat were immediately removed, rinsed with a physiological saline solution, weighed, and then frozen in liquid nitrogen. All samples were stored at  $-70^\circ\text{C}$  until analyzed. The study protocol was approved by the Ethics Committee of College of Biosystems Engineering and Food Science, Zhejiang University.

### Histological observation of liver and WAT

The specific part of liver and perirenal WAT were removed from the rats, rinsed with saline and fixed in a buffer solution of 10% formalin. Sections of fixed tissue specimens were processed for paraffin embedding, and 4- $\mu\text{m}$  sections were prepared and stained with hematoxylin-eosin and observed under the light microscopy (OLYMPUS BX41) with the magnifying power of 100 $\times$  and 200 $\times$ .

### Analysis of serum lipid profile

Concentrations of serum total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and glucose were analyzed on HIT-ACHI 7020 chemistry analyzer using colorimetric test supplied by Diasys Diagnostic Systems (Shanghai) Co., Ltd., China.

### Determination of serum/plasma insulin, leptin, ghrelin and obestatin levels

Serum insulin and leptin, and plasma ghrelin and obestatin concentrations were analyzed by Rat Insulin (INS) ELISA kit, Rat Leptin ELISA kit, Rat Growth hormone releasing peptide-Ghrelin (GHRP-Ghrelin) ELISA kit and Rat Obestatin ELISA kit, respectively (Nanjing Jiancheng Technology Co., LTD., China).

### RNA extraction and quantitative real-time RT-PCR analysis

Total RNA was extracted from perirenal WAT using TRIZOL reagent (Takara Biotechnology Co., LTD., China) according to the manufacture's instructions. The concentrations of RNA samples were measured and quantified spectrophotometrically (Thermo Scientific NanoDrop 2000c Spectrophotometer). Then, cDNA was synthesized from total RNA using the PrimeScript RT reagent kit (Takara Biotechnology Co., LTD., China). Real-time quantitative RT-PCR analysis was performed with an automated sequence detection system (BIO-RAD, CFX96). The mRNA expression of adiponectin, leptin, adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), carnitine palmitoyltransferase 1A (CPT1A), PPAR $\gamma$ , and uncoupling protein 2 (UCP2) in WAT was measured by quantitative real-time RT-PCR using SYBR green PCR reagents (Takara Biotechnology Co., LTD., China). PCR rat primers were used for adiponectin, 5'-GGAACTGTGTCAGGTTGGATG-3' (forward), 5'-GGGTCACCTTAGGACCAAGAA-3' (reverse); leptin, 5'-TTCAAGCTGT GCCTATCCACAAAG-3' (forward), 5'-TGAAGCCCGGGAATGAAGTC-3' (reverse); ATGL, 5'-TGACTCGAGTTTCGGATGGA-3' (forward), 5'-GAAATGCCGCCATCCA CATAG-3' (reverse); HSL (lipe), 5'-CTGGAGTTAAGTGGGCGCAAG-3' (forward), 5'-CA GAC-ACACTCTGCGCATAGAC-3' (reverse); LPL, 5'-GCCAGCAACAT-TATCCAGT GTC-3' (forward), 5'-AGCAGCATGGGCTCCAAGA-3' (reverse); CPT1A, 5'-CGCTCATG GTCAACAGCAACTAC-3' (forward), 5'-TCACGGTCTAATGTGCGACGA-3' (reverse); PPAR $\gamma$ , 5'-TGTCGGT-TTCAGAAGTGCTTG-3' (forward), 5'-TTCAGCTGGTCGATAT CACTG-GAG-3' (reverse); UCP2, 5'-GCTGGTGACCTATGACCTCATCAA-3' (forward), 5'-GTACTGGCCCAAGGCAGAGTTC-3' (reverse). The quantitative endpoint for real-time PCR is the threshold cycle (CT), which defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold. The fold changes were calculated using the  $2^{-\Delta\Delta\text{CT}}$  method with  $\beta$ -actin as the internal control gene [23].

### Statistical analysis

Results are expressed as means  $\pm$  standard error of the mean (SE). The data were analyzed with a one-way ANOVA, followed by LSD and Duncan's test. Differences with  $P < 0.01$  or  $P < 0.05$  were considered significant.

## Results and discussion

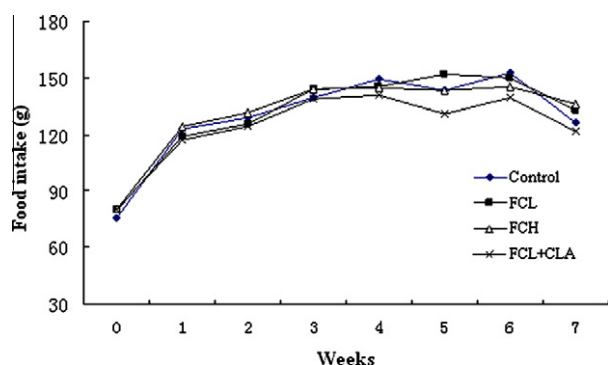
### Body weight, food intake and adipose tissue weight

Currently, dietary fat is one of most commonly used environmental factors associated with the induction of obesity in rodents. In the present study, high-fat was used in the diet to induce obesity

of SD rats. Maeda et al. [24] reported that 0.2% Fc significantly attenuated the body weight gain and WAT weight of diet-induced obese mice relative to the control mice. Recently, Woo et al. showed that 0.05% and 0.2% Fc both significantly suppressed body weight gain reaching 15% and 19%, respectively, in C57BL/6 N mice fed 20% high-fat [6]. Likewise, the present study demonstrated that high-fat diet supplemented with FCL + CLA resulted in a significant reduction in body weight gain ( $P < 0.05$ ) and WAT weight gain ( $P < 0.05$ ) compared with control group without affecting food intake (Fig. 1, Table 1 and 2). WAT weight of FCL and FCH rats was also inhibited compared to control group ( $P < 0.05$ ), though body weight was not affected by Fc supplement, which is possibly attributed to the low levels of Fc (less than 0.2% in diet) and/or short-term treatment. WAT is a primary site of energy storage in the form of triacylglycerol droplets, and it accumulates triacylglycerols during nutritional excess [25]. Furthermore, according to the report of Hosokawa et al. [26], 2.2% seaweed lipids containing Fc attenuated WAT weight gain of C57BL/6 J mice by feeding of 30% high-fat diet. In the present study, relative weights of adipose tissues were summarized in Table 2. Mesenteric WAT weights were significantly attenuated in FCL, FCH and FCL + CLA groups in comparison with control group whereas perirenal and inguinal WAT weights were markedly lowered only in FCL + CLA rats ( $P < 0.05$ ). Nevertheless, total WAT weight of rats in FCL, FCH and FCL + CLA groups was all significantly suppressed relative to control rats ( $P < 0.05$ ). Maeda et al. [5] reported that brown adipose tissue (BAT) weight was significantly greater in 2.0% *Undaria* lipid-fed mice than in control mice. However, in the present study, there was no difference in BAT weight among all groups, which may be due to the little amount of BAT in rats and the lower dose of Fc supplemented in the diet. In addition, it was confirmed that a positive correlation between body weight and visceral fat weights exists (perirenal:  $r = 0.840$ ,  $P < 0.01$ ) [6]. In the present study, a positive correlation also existed between body weight and perirenal WAT weight ( $r = 0.704$ ,  $P < 0.01$ ). As such, the body weight loss observed in FCL + CLA group was partly due to a decrease in WAT weight.

#### Histology of liver and adipose tissue

Until recently, lipid droplets were viewed as an inert storage pool of TG [27]. Additionally, dietary CLA isomers reduced lipid accumulation in hepatic cells, hence contributing to lipid droplet maintenance or lipolysis [28]. Likewise, in the present study, the number of lipid droplets in liver cells was apparently diminished in rats fed FCL, FCH and FCL + CLA in comparison with control rats



**Fig. 1.** Food intake of high-fat fed rats. Mean  $\pm$  SE,  $n = 5$  for Control group, and  $n = 10$  for FCL, FCH and FCL + CLA groups, respectively. FCL, 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCH, 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCL + CLA, combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

**Table 1**

Effects of FCL, FCH and FCL + CLA supplementation on body weight in high-fat fed rats.

	Control	FCL <sup>a</sup>	FCH <sup>b</sup>	FCL + CLA <sup>c</sup>
Initial body weight (g)	147 $\pm$ 5.72	148 $\pm$ 4.32	156 $\pm$ 4.37	142 $\pm$ 5.55
Final body weight (g)	522 $\pm$ 11.37	512 $\pm$ 5.04	531 $\pm$ 7.34	485 $\pm$ 9.08**
Body weight gain (g)	375 $\pm$ 10.15	363 $\pm$ 5.81	375 $\pm$ 8.80	344 $\pm$ 11.91*

Mean  $\pm$  SE,  $n = 5$  for Control group, and  $n = 10$  for FCL, FCH and FCL + CLA groups, FCL + CLA vs. Control.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

<sup>a</sup> 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>b</sup> 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>c</sup> Combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

(Fig. 2), moreover, liver weight of FCL + CLA rats was markedly lowered by 17.3% relative to control rats ( $P < 0.05$ ) (Table 2). Accordingly, Fc and FCL + CLA supplementation had a pleasant alleviation on the accumulation of lipid droplets in liver cells.

Obesity is characterized at the cell biological level by an increase in the number and size of adipocytes differentiated from fibroblastic pre-adipocytes in the adipose tissue [29]. The present study revealed that perirenal adipocyte sizes of FCL, FCH and FCL + CLA groups were smaller than those of control group (Fig. 3).

#### Serum lipid profile, and levels of leptin, ghrelin and obestatin

The current study indicated that FCL, FCH and FCL + CLA supplementation was effective in improving serum lipid profile via significantly affecting TG, TC and glucose levels, and maintaining a modest reduction in LDL-C level, though HDL-C concentrations were apparently reduced in rats fed FCH and FCL + CLA (Table 3). Many studies have clarified that leptin secretions are elevated through the accumulation of fat in adipocytes and causes insulin resistance in obese animal models [30]. Therefore, plasma/serum leptin level is used as an index of body fat accumulation. Here, two doses of Fc and FCL + CLA significantly decreased the serum leptin concentration compared with control group ( $P < 0.01$ ) (Table 4). Further, the leptin level in serum exhibited a positive correlation with WAT weight ( $r = 0.571$ ,  $P < 0.01$ ) (data not shown). Asakawa et al. reported that exogenous ghrelin stimulates food intake and promotes energy storage [31]. Additionally, ghrelin treatment in rodents leads to sustained gain of fat mass induced via regulating food intake, decreased fat oxidation rates and thermogenesis, and increased lipogenesis in adipose tissue [32]. In contrast, obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake, hence decreasing body-weight gain [33]. In the present study, there were no differences in plasma levels of ghrelin and obestatin, however, a slight descending trend was observed in ghrelin levels in FCH and FCL + CLA groups and a mild increasing trend was revealed in obestatin levels in FCL and FCH groups (Table 4).

#### mRNA expression of lipid-regulating enzymes in adipose tissue

Currently, it is accepted that specific regulation of gene expression in adipocytes is one of the most important targets for the intervention of obesity. In addition, WAT has been regarded as an active endocrine organ which secretes important molecules, like leptin and adiponectin, involved in the regulation of body weight [34]. Adiponectin is a secreted protein which plays a major role in the regulation of glucose, insulin and fatty acids and which has an anti-obesity effect [35,36]. In addition, adiponectin is very highly expressed in adipose tissues and it can increase  $\beta$ -oxidation

**Table 2**  
Effects of FCL, FCH and FCL + CLA supplementation on WAT, BAT and liver weights in high-fat fed rats.

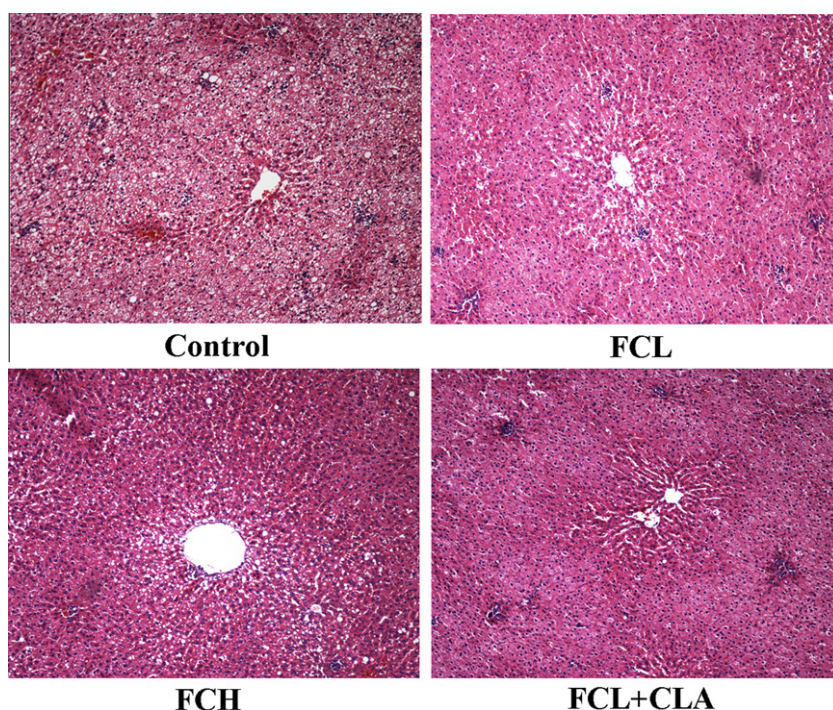
	Control	FCL <sup>A</sup>	FCH <sup>B</sup>	FCL + CLA <sup>C</sup>
Epididymal WAT (g)	2.12 ± 0.25	1.93 ± 0.11	1.87 ± 0.11	1.78 ± 0.13
Perirenal WAT (g)	6.18 ± 0.95 <sup>a</sup>	5.66 ± 0.44 <sup>abc</sup>	6.23 ± 0.57 <sup>ab</sup>	4.61 ± 0.37 <sup>bcd</sup>
Mesenteric WAT (g)	8.36 ± 0.55 <sup>a</sup>	4.27 ± 0.44 <sup>b</sup>	5.10 ± 0.30 <sup>b</sup>	3.82 ± 0.38 <sup>b</sup>
Inguinal WAT (g)	2.66 ± 0.46 <sup>a</sup>	2.07 ± 0.14 <sup>abc</sup>	2.37 ± 0.19 <sup>ab</sup>	1.59 ± 0.10 <sup>c</sup>
Interscapular BAT (g)	0.417 ± 0.043	0.448 ± 0.031	0.422 ± 0.033	0.489 ± 0.046
Total WAT (g)	18.79 ± 1.17 <sup>a</sup>	11.16 ± 0.38 <sup>b</sup>	12.87 ± 1.15 <sup>b</sup>	10.99 ± 0.60 <sup>b</sup>
Liver weight (g)	20.2 ± 1.38 <sup>a</sup>	17.9 ± 0.82 <sup>a</sup>	18.9 ± 1.17 <sup>a</sup>	16.7 ± 0.76 <sup>b</sup>

Mean ± SE, *n* = 5 for Control group, and *n* = 10 for FCL, FCH and FCL + CLA groups, <sup>abc</sup>Means in the row not sharing a common letter are significantly different between groups at *P* < 0.05 as determined by a one-way ANOVA test.

<sup>A</sup> 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>B</sup> 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>C</sup> Combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

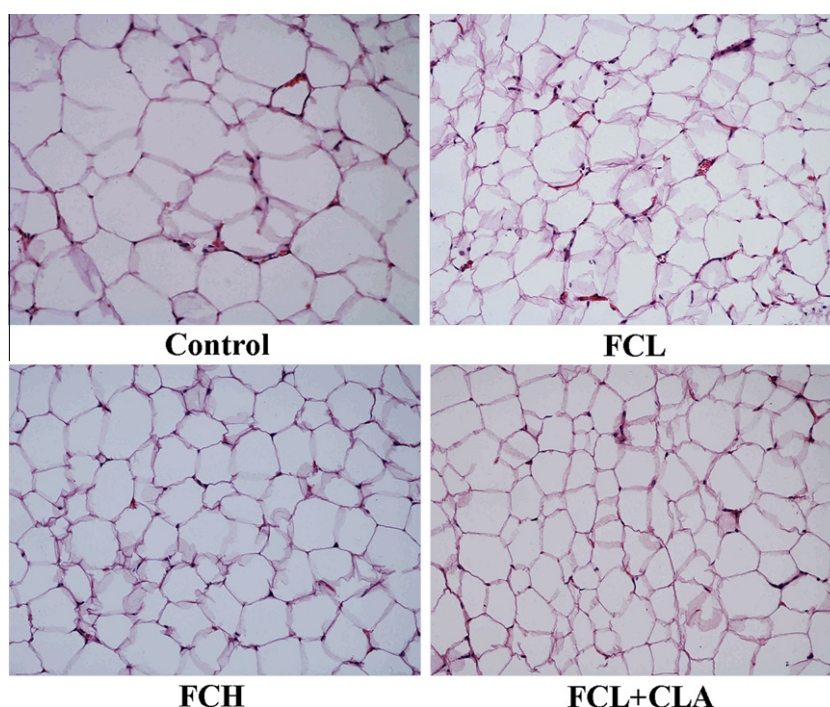


**Fig. 2.** Histological features on liver in high-fat fed rats (100×). Liver tissue of SD rats was stained with hematoxylin and eosin. FCL, 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCH, 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCL + CLA, combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

in tissues and causes weight loss in mice [37]. Moreover, adiponectin levels are inversely related with fat mass [38]. In the present study, the mRNA expression of adiponectin was remarkably elevated in FCL, FCH and FCL + CLA groups in perirenal WAT compared to control group (*P* < 0.05) (Fig. 4). So far, a large body of work proposed that leptin exerts its actions on food intake and energy expenditure. However, in the present study, no significant difference was observed in the mRNA expression of leptin in perirenal WAT among all groups.

Deregulation of lipid metabolism has long been recognized as an essential factor in the development of obesity and WAT lipolysis plays a pivotal role in controlling the quantity of TG stored in fat depots [39]. Reports showed that ATGL is the rate-limiting enzyme for the first step in TG hydrolysis, generating diacylglycerol (DG) and fatty acid (FA), whereas HSL is responsible for the subsequent degradation of DG, generating MG and FA [28]. Apart from ATGL and HSL, LPL is another enzyme, rate-limiting for the hydrolysis of core TGs in chylomicrons and VLDLs [40,41]. Furthermore, adipose LPL hydrolyzes the TG of lipoprotein particles in capillaries, thereby releasing FA and then transported into adipocytes where

they are esterified to TG and stored for future energy use [42]. Elevated LPL in adipocytes thus promotes the storage of excess FFA in adipose tissue [43]. In the present study, the up-regulation of ATGL mRNA in perirenal WAT was observed in FCL, FCH and FCL + CLA groups (*P* < 0.05). Further, FCL and FCH also enhanced mRNA expression of HSL (*P* < 0.05) and LPL (*P* < 0.01) respectively, which were predominantly involved in hydrolyzing triacylglycerols. These results suggest that the hydrolysis of excessive triacylglycerols stimulated by FCL, FCH and FCL + CLA contributed to diminishing the fat stores and combating obesity. The enzyme CPT1A regulates the entry of LCFAs into mitochondria, where they undergo β-oxidation [44,45]. The present study showed that mRNA expression of CPT1A in perirenal WAT of rats fed FCL, FCH and FCL + CLA was all significantly increased compared with control rats (*P* < 0.01, *P* < 0.05), which supports the hypothesis that the enhancement of fatty acids mobilization and oxidation was potentially triggered by Fc and CLA. Moreover, PPARγ, a regulator of adipogenic gene expression, was significantly down-regulated by FCH and FCL + CLA (*P* < 0.05). PPARs are nuclear hormone receptors that control lipid oxidation, adipocyte differentiation, glucose and lipid



**Fig. 3.** Histological features on perirenal adipose tissue in high-fat fed rats (200 $\times$ ). Perirenal adipose tissue of SD rats was stained with hematoxylin and eosin. FCL, 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCH, 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCL + CLA, combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

**Table 3**

Effects of FCL, FCH and FCL + CLA supplementation on TC, TG, HDL-C, LDL-C and glucose levels in high-fat fed rats.

	Control	FCL <sup>A</sup>	FCH <sup>B</sup>	FCL + CLA <sup>C</sup>
TC (mmol/L)	2.15 $\pm$ 0.081 <sup>a</sup>	1.88 $\pm$ 0.144 <sup>ab</sup>	1.39 $\pm$ 0.067 <sup>b</sup>	1.61 $\pm$ 0.107 <sup>ab</sup>
TG (mmol/L)	1.23 $\pm$ 0.064 <sup>a</sup>	0.76 $\pm$ 0.046 <sup>b</sup>	0.54 $\pm$ 0.022 <sup>b</sup>	0.48 $\pm$ 0.036 <sup>b</sup>
HDL-C (mmol/L)	0.71 $\pm$ 0.035 <sup>a</sup>	0.62 $\pm$ 0.012 <sup>a</sup>	0.45 $\pm$ 0.049 <sup>b</sup>	0.37 $\pm$ 0.031 <sup>b</sup>
LDL-C (mmol/L)	0.51 $\pm$ 0.058	0.37 $\pm$ 0.035	0.44 $\pm$ 0.036	0.28 $\pm$ 0.027
Glucose (mmol/L)	4.56 $\pm$ 0.242 <sup>a</sup>	3.94 $\pm$ 0.202 <sup>ab</sup>	3.80 $\pm$ 0.090 <sup>b</sup>	3.79 $\pm$ 0.259 <sup>b</sup>

Mean  $\pm$  SE,  $n$  = 5 for Control group, and  $n$  = 10 for FCL, FCH and FCL + CLA groups.

<sup>abc</sup>Means in the row not sharing a common letter are significantly different between groups at  $P$  < 0.05 as determined by a one-way ANOVA test.

<sup>A</sup> 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>B</sup> 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>C</sup> Combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

**Table 4**

Effects of FCL, FCH and FCL + CLA supplementation on serum insulin and leptin, plasma ghrelin and obestatin levels in high-fat fed rats.

	Control	FCL <sup>1</sup>	FCH <sup>2</sup>	FCL + CLA <sup>3</sup>
INS (mU/L)	2.35 $\pm$ 0.08	2.40 $\pm$ 0.13	2.19 $\pm$ 0.13	2.11 $\pm$ 0.06
LEP ( $\mu$ g/L)	2.33 $\pm$ 0.17 <sup>a</sup>	1.60 $\pm$ 0.06 <sup>b</sup>	1.55 $\pm$ 0.05 <sup>bc</sup>	1.41 $\pm$ 0.04 <sup>c</sup>
GHRE (ng/L)	236.59 $\pm$ 18.30	234.71 $\pm$ 21.13	219.55 $\pm$ 10.06	201.36 $\pm$ 11.24
OBES (ng/L)	70.09 $\pm$ 4.03	83.91 $\pm$ 8.21	79.90 $\pm$ 4.04	68.96 $\pm$ 3.51

Mean  $\pm$  SE,  $n$  = 5 for Control group, and  $n$  = 10 for FCL, FCH and FCL + CLA groups. <sup>abc</sup>Means in the row not sharing a common letter are significantly different between groups at  $P$  < 0.01 as determined by a one-way ANOVA test. INS, insulin; LEP, leptin; GHRE, ghrelin; OBES, obestatin.

<sup>1</sup> 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

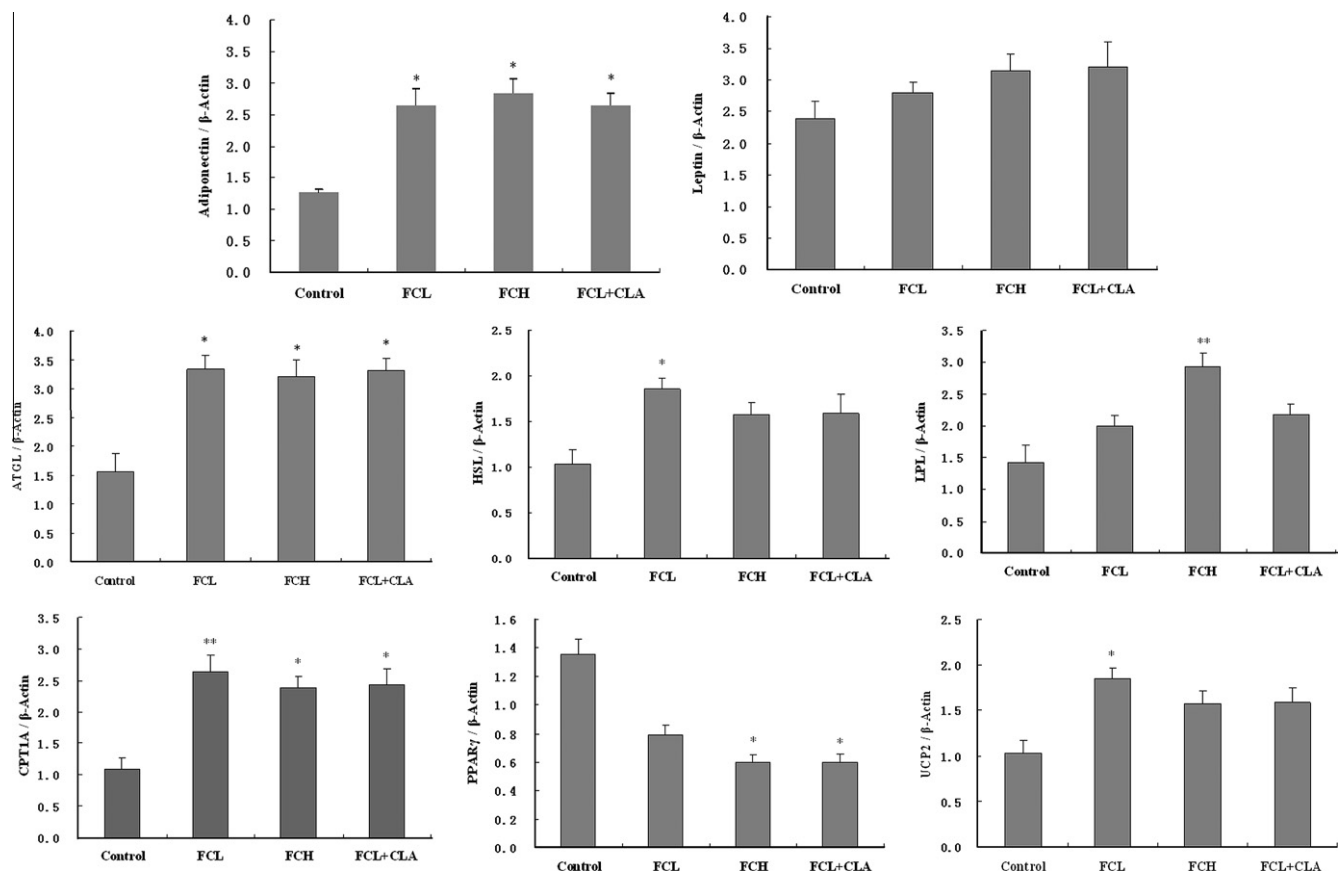
<sup>2</sup> 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet.

<sup>3</sup> Combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

storage, and inflammation [46,47]. Regulation of PPAR $\gamma$  would be one of the expected mechanisms underlying the anti-obesity effect of dietary Fc and CLA.

Evidence to date indicates that the product of the UCP2 gene is crucial for mammalian thermogenesis because of its high degree of sequence similarity (55–60%) to UCP1 [48]. UCP2 is widely expressed in human and rodent tissues [48–51], unlike UCP1, which

is expressed uniquely in BAT. The ubiquitous expression of UCP2 suggests that the protein may be important for determining basal metabolic rate, and possibly regulating body weight in mammals including humans [52]. In the present study, compared with control group, there was a significant elevation of UCP2 mRNA expression in perirenal WAT in FCL group ( $P$  < 0.05), which suggest a potential contribution to the underlying thermogenesis.



**Fig. 4.** Gene expressions of adipocytokines and lipid-regulating enzymes in perirenal WAT using quantitative RT-PCR analysis. Mean  $\pm$  SE,  $n = 5$  for Control group, and  $n = 10$  for FCL, FCH and FCL + CLA groups. mRNA was normalized to  $\beta$ -Actin and expressed as a ratio to the corresponding amount in the control group. \* $P < 0.05$ , \*\* $P < 0.01$  vs. Control. ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; CPT1A, carnitine palmitoyltransferase 1A; UCP2, uncoupling protein 2. FCL, 0.083 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCH, 0.167 mg/kg/bw fucoxanthin-supplemented group with a high-fat diet; FCL + CLA, combination of 0.083 mg/kg/bw fucoxanthin and 0.15 g/kg/bw CLA-supplemented group with a high-fat diet.

## Conclusions

The present study showed that Fc reduced WAT weight while FCL + CLA decreased both body weight and WAT weight in rats. Both Fc and FCL + CLA could reduce serum concentration of TG, glucose and leptin. The mechanism underlying the anti-obesity effect of FCL + CLA may be elucidated through up-regulating expression of adiponectin, ATGL, CPT1A and down-regulating mRNA expression of PPAR $\gamma$  in WAT, which are involved in  $\beta$ -oxidation of fatty acids and triacylglycerol hydrolysis, however, the profound synergistic relationship between Fc and CLA need to be clarified by further study.

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