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Antiobesity effect of PEGylated conjugated linoleic acid on high-fat diet-induced obese C57BL/6J (*ob/ob*) mice: attenuation of insulin resistance and enhancement of antioxidant defenses

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Abstract

This study was designed to test that dietary conjugated linoleic acids (CLA) used in a mixture of *cis*-9,*trans*-11 and *trans*-10, *cis*-12 isomers (40% each in weight) coupled to poly(ethylene glycol) (PEG) as PEGylated CLA (PCLA) act as mediators inducing or inhibiting specific metabolic pathways in high-fat (HF)-fed obese C57BL/6J (*ob/ob*) mice. After an acclimatization period of 7 days, animals were given a normal (control) or HF diet, the latter being added either alone (HF) or with CLA, PEG or PCLA for 6 weeks. Although the food intakes were not different among the dietary groups, final body weights were significantly lower in the HF-PCLA group than in the HF group. Also the HF-PCLA diet strongly prevented the dramatic increase in blood low-density lipoprotein cholesterol observed with the HF diet, with no difference in high-density lipoprotein cholesterol between control, HF and HF-PCLA treatments. Furthermore, homeostasis model assessment levels showed a marked decrease in HF-PCLA-fed mice, preventing the increase found in mice fed the HF diet, and suggesting that PCLA lowered insulin resistance in HF-mice. The liver steatosis observed in mice fed the HF diet was also prevented by PCLA. Interestingly, the activity of mitochondrial glutathione peroxidase was increased by PCLA, which may enhance antioxidant defenses. Overall, PCLA exerted its beneficial effects through reduction of lipid accumulation and attenuation of insulin resistance induced by the HF diet in obese C57BL/6J (*ob/ob*) mice, which might confer to these products antiobesity properties in other species.

Keywords: PEGylated conjugated linoleic acid; C57BL/6J (*ob/ob*) mouse; Lipid reduction; Insulin resistance; Anti-oxidant defense

1. Introduction

Obesity is the major risk factor for numbers of disorders including hypertension, hyperlipidemia and heart diseases [1,2]. Also, type 2 diabetes is characterized by impaired glucose and lipid metabolism and is associated with obesity [2]. Generally, consumption of high levels of dietary fat is thought to be a major factor in promoting obesity in humans as well as in animal models [3]. Increasing evidence suggested that some of the adipose tissue-derived molecules are involved in the pathophysiology of obesity-related insulin resistance and atherosclerosis [4]. For these reasons, many scientists have highlighted the importance of gaining a better understanding of this epidemic with the aim of developing effective therapies. Unfortunately, few therapeutic agents are available for the treatment of obesity.

It has been shown that *trans*-10,*cis*-12 conjugated linoleic acid (CLA) or a mixture of *trans*-10,*cis*-12 and

Abbreviations: AF, abdominal fat; CLA, conjugated linoleic acid; C/EBPα, CCAAT/enhancer-binding protein alpha; FAS, fatty acid synthase; FFA, free fatty acid; GPDH, glycerol-3-phosphate dehydrogenase; GPX, glutathione peroxidase; GSH, glutathione; HDLC, high-density lipoprotein cholesterol; HF, high fat; HOMA, homeostasis model assessment; LDLC, low-density lipoprotein cholesterol; PCLA, PEGylated CLA; PEG, poly (ethylene glycol); PPARγ, peroxisome proliferator-activated receptor gamma; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride.

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cis-9,*trans*-11 isomers reduced body fats in vitro and in vivo [5–8]. CLAs were given experimental models as free form or esterified in triglycerides [6,7], but their efficiency may be improved through chemical or physical modification of CLA to enhance their solubility in water and to increase the clinical application [9,10]. Poly(ethylene glycol) (PEG) has been found to increase solubility in water and reduce secondary aggregation of the liposomes [11]. Also, the Food and Drug Administration has approved PEG for human intravenous, oral and dermal applications [12]. In fact, the advantages of PEGylation include improved circulation time in vivo, reduced antigenicity and immunogenicity, improved bioavailability and reduced toxicity [13]. In addition, PEG can be eliminated through a combination of renal and hepatic pathways, thus making it ideal for pharmaceutical applications [14].

Generally, C57BL/6J (*ob/ob*) models have been used for investigation of human obesity and metabolic syndrome [15]. When raised on a low-fat diet, the C57BL/6J (*ob/ob*) mice are lean and euglycemic with normal insulin levels and blood pressure. However, when raised on a high-fat (HF) diet, animals develop central adiposity, hyperinsulinemia, hyperglycemia and hypertension [16]. These syndromes appear to be related to abnormalities in adrenergic control of adipocyte function, which, in turn, appear to be related to hyperinsulinemia [16]. Also, the development of insulin resistance, hyperglycemia and obesity in the C57BL/6J (*ob/ob*) mice closely parallel the progression of common forms of the human diseases [17].

We previously reported that PEGylated CLA (PCLA) increased solubility in water and promoted bioavailability, by reference to the free fatty acid (FFA) form, due to the biocompatible and hydrophilic properties of PEG [18]. Based on our previous in vitro observations, this study was designed to investigate whether PCLA was as efficient as CLA for inducting or inhibiting, in obese C57BL/6J (*ob/ob*) mice fed a HF diet, metabolic pathways related to lipid metabolism.

2. Materials and methods

2.1. Materials

PEG (MW 2,000) was provided by Aldrich (Milwaukee, WI, USA). A mixture of CLAs (*trans*-10,*cis*-12, *cis*-9,*trans*-11 and other CLA isomers, 40/40/20 in wt, respectively) was provided by Lipozen (Gyeonggi, South Korea). CLA isomers of the mixture were coupled to PEG by using our previous PEGylation method [18]. The PCLA was dissolved in *d*-chloroform, and the composition of CLA in the PCLA was estimated by ^{1}H nuclear magnetic resonance (^{1}H NMR) (Bruker, Avance 500) to be 65 mol%.

2.2. Experimental design

The protocol for this study was approved by the Animal Care and Use Committee of Seoul National University. Five-week-old male C57BL/6J (*ob/ob*) mice purchased from Central Lab. Animal (Seoul, South Korea) were housed individually in a room with controlled temperature (20–22°C), humidity (55–60%) and lighting (9:00–21:00 h). They were fed a commercial AIN-93G diet (HanSam R&D, Gyeonggi, South Korea) over 7 days. After this time of acclimatization, mice were randomly divided into five groups (n=10), each receiving the normal diet (control group) or a HF diet with the initial composition or containing 1% PEG (HF-PEG) or CLA (HF-CLA) or PCLA (HF-PCLA) over 6 weeks. Dietary intake and body weight were recorded every 3 days.

2.3. Blood analysis

All mice were sacrificed after an overnight 12-h fast. Blood was collected by orbital venipuncture. The blood was allowed to coagulate and centrifuged at 3000 rpm for 15 min, and serum was stored at −20°C until analysis. Triglyceride (TG), total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol (HDLC) were enzymatically analyzed using a commercial kit (Asan Pharmaceutical, Seoul, South Korea). Low-density lipoprotein (LDL) cholesterol (LDLC) was calculated from TG, TC and HDLC concentrations using the following Friedewald formula [19]: LDLC=TC −[(HDLC−TG)/5]. The insulin resistance index from plasma insulin and glucose was estimated by the HOMA parameter: HOMA=plasma insulin (μU/ml)×plasma glucose (mM)/22.5; as seen from the equation, the greater the HOMA value, the greater the level of insulin resistance [20].

2.4. Tissue analysis and activity

2.4.1. Liver antioxidant defenses

Immediately after sacrifice, mitochondrial fractions were prepared from liver as in [21]. Activities of gluthatione (GSH) peroxidase (GPX), GSH reductase and GSH contents were measured by spectrophotometry using diagnostic EIA kits (Bioxytech GPX-340, Bioxytech GR-340 and Bioxytech GSH-400, respectively) obtained from Oxis Research (Portland, OR, USA). Activities of superoxide dismutase (SOD) in liver homogenates and of manganese SOD in mitochondrial fractions were measured using the Bioxytech SOD-525 kit. Malondialdehyde (MDA) was measured according to the method of Okhawa et al. [22].

2.4.2. Adipose tissue activities

Fatty acid synthase (FAS) and glycerol-3-phosphate dehydrogenase (GPDH) were measured with commercial kits (Takara, Kyoto, Japan) using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). One unit of enzyme activity represents 1 nmol of NADH oxidised per min. Protein concentration was measured with the Bradford method [23].

2.4.3. Carcass composition

The frozen eviscerated carcasses were homogenized in distilled water using a Polytron (PRO300D PRO; Scientific).

Table 1
Composition of experimental diets

Formula	AIG-93G	HF	HF-PEG (g%)	HF-CLA	HF-PCLA
Protein	20	20	20	20	20
Carbohydrate	64	46	46	46	46
Fat	7	24	24	24.2	24.2
Ingredient			g/kg		
Casein	200	200	200	200	200
L-Cystine	3	3	3	3	3
Corn starch	397	224	224	224	224
Sucrose	100	100	100	100	100
Dextrose	132	132	132	132	132
Cellulose	50	50	50	50	50
Soybean oil	70	70	70	70	70
Lard	0	173	173	173	173
TBHQ	0.014	0.014	0.014	0.014	0.014
Mineral mix	35	35	35	35	35
Vitamin mix	10	10	10	10	10
Choline bitartrate	3	3	3	3	3
PEG	–	–	28	–	–
CLA [a]	–	–	–	10	–
PCLA [b]	–	–	–	–	38

PEG, CLA and PEG-CLA (PCLA) were mixed to a basal AIN-93G diet enriched in fat (HF).
TBHQ, *t*-butylhydroquinone.
[a] CLA isomers (g/10g) containing: *trans*-10,*cis*-12 CLA 4, *cis*-9,*trans*-11 CLA 4, other isomers 2.
[b] CLA isomers in PCLA (g/38g) containing: *trans*-10,*cis*-12 CLA 4, *cis*-9,*trans*-11 CLA 4, other isomers 2, PEG 28.

Homogenates were dried to constant weight in a 70°C oven and then finely ground and mixed thoroughly using a mortar and pestle. The final product was analyzed in triplicate for lipid, protein and ash content. Lipid was analyzed using a Soxtec System HT 1043 Extraction unit using 2:1 chloroform-methanol, protein with a Perkin-Elmer Series II Nitrogen Analyzer 410 and ash by a CEM MAS 7000 Microwave Muffle Furnace. Water content of the carcass was calculated by subtracting the dried carcass weight from the original weight of the eviscerated carcass.

2.4.4. Protein expression

For Western blot analysis, 500 μg of AF samples were homogenized in 1 ml of lysis buffer (20 mM Tris, 145 mM NaCl, 10% glycerol, 5 mM EDTA, 1% Triton-X, 0.5% Nonidet P-40, 100μM phenylmethylsulfonyl fluoride, 50 μM NaF, 1 mM sodium orthovanadate). Lysates were centrifuged at 8850*g* at 4°C for 10 min. The upper lipid phase was removed from adipose tissue samples, and they were recentrifuged under the same conditions. The supernatant was collected, and the protein concentration was determined by the Bradford method [23], using bovine serum albumin as a standard. Fifty micrograms of protein were loaded in each lane. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were blotted onto poly(vinylidene difluoride) (PVDF) membranes (Amersham Pharmacia Biotech., Buckinghamshire, England) using a Kem-En-Tec semidry blotter. Transfer of protein to PVDF was carried out on the same day as the protein harvest to prevent freeze–thaw degradation of phosphoproteins. After transfer, the membranes were blocked overnight in TBS containing 5% defatted dry milk and 0.1% Tween 20 (Biosesang, Seoul, Korea). Incubation with primary and secondary antibodies was performed in TBS containing 5% defatted nonfat dry milk and 0.1% Tween 20 (TBS-T) for 2 h. After incubation with antibodies, membranes were washed in TBS containing 0.1% Tween 20. The primary antibodies used (2000:1 primary antibodies-TBS-T) were mouse monoclonal peroxisome proliferator-activated receptor gamma (PPARγ) antibody recognizing both PPARγ isoforms and mouse monoclonal antibody against mouse CCAAT/enhancer-binding protein alpha (C/EBPα). Secondary antibody used (3000:1 secondary antibody-TBS-T) was horseradish peroxidase-conjugated antimouse antibody (Santa Cruz Biotech, San Francisco, CA, USA). Enhanced chemiluminescence (ECL) (Intron Biotech., Seoul, South Korea) was used for detection. Measurement of signal intensity on PVDF membranes after Western blotting with various antibodies was performed using a FluorChem densitomer with the Alpha-Ease-FC image processing and analysis software (Alpha Innotech). After normalization to GAPDH protein, density values for the protein bands of interest were expressed as percentage of the control. All figures showing quantitative analysis include data from at least five independent experiments.

2.5. Statistical analysis

Data were expressed as compared to the control (HF-fed animals) and were expressed as means (n=10)±S.D. Data were analyzed using one-way analysis of variance (ANOVA) followed by paired Student's t tests for multiple comparisons. Western blot data were analyzed by using Student's t test. Statistical significance was represented by ∗ for P values less than .05 and by ∗∗ for P values less than .01.

3. Results

3.1. Experimental design

With diets containing 0.25–2.0% CLA, there was a dose-dependent decrease in body fat content and adipose tissue weight in various experimental animals [5,7]. Also, CLA greater than 0.5% was necessary to obtain a clear-cut suppression of body fat content, and most studies used a 1% CLA diet to examine the physiological activity of CLA [24–26]. For this reason, we used 1% CLA in the HF diet (Table 1). Also, as indicated in the Materials and methods section, the CLA in the PCLA mixture was estimated by ^{1}H NMR to be 65 mol%. The amount of PCLA added to diets was therefore corrected to be 1% CLA in the HF diet. The observations that all results obtained with or without PEG were totally comparable led us to limit this repetition. In tables and figures, HF is indicated (added or not with PEG), and the same for HF-CLA, but this systematic repetition has

Table 2
Body weight and food intake in mice fed HF diets containing CLA

	Normal	HF (added or not with PEG)	HF-CLA (added or not with PEG)
Body weight (g)			
Initial	20.7±0.4	20.3±0.8	20.1±0.3
Final	27.9±1.8^{b,c}	34.7±1.2^{a}	25.8±2.2^{c}
Food intake (g/day)	4.3±1.9	4.2±0.5	4.2±1.3
Feed efficiency	0.032±0.004	0.081±0.007^{a}	0.031±0.002^{b}
Weight gain (g/day)	0.17±0.08b^{c}	0.34±0.05^{a}	0.13±0.04^{c}

Data were analyzed using one-way ANOVA followed by paired Student's *t* tests for multiple comparisons. Values (means±S.D.; *n*=10) not sharing a common superscript differ significantly (*P*<.05).

been totally omitted from the text in the Results and Discussion sections.

3.2. Body weight and food intake

After 6 weeks, HF-fed mice weighed nearly 8.9 g more than HF-CLA-fed mice (Table 2). In contrast, the final body weights were significantly reduced in the HF-CLA dietary groups compared to in the HF dietary groups.

3.3. Organ weights

The HF diet had no effect on spleen, kidney, or lung weights when compared to the normal and HF-CLA diet (Table 3). However, abdominal fat (AF) weights were significantly greater in the HF groups than in the other ones. By contrast, liver and AF weights of mice fed HF-CLA were significantly lower than of those fed the normal or HF diets.

3.4. Carcass composition

The HF-CLA-fed animals had an average of 31.57 g carcass lipid compared to 25.32 g in HF-CLA-fed mice (Table 4). Carcass protein, water and ash contents were barely increased in the HF-CLA dietary groups when compared to the HF dietary groups. By contrast, there was no difference in water and ash contents between HF-CLA and normal dietary groups.

Table 3
Organ weights in mice fed HF diets containing CLA

	Normal	HF (added or not with PEG)	HF-CLA (added or not with PEG)
Organ weight (g)			
Liver	1.403±0.321^{a,b}	1.643±0.312^{a}	1.210±0.320^{b}
Spleen	0.065±0.072	0.067±0.021	0.068±0.072
Kidney	0.065±0.013	0.061±0.019	0.060±0.017
Lung	0.109±0.044	0.109±0.097	0.110±0.017
AF	0.354±0.072^{b,c}	0.671±0.033^{a}	0.292±0.032^{c}

Data were analyzed using one-way ANOVA followed by paired Student's *t* tests for multiple comparisons. Values (means±S.D.; n=10) not sharing a common superscript differ significantly (*P*<.05).

Table 4
Carcass composition in mice fed HF diets containing CLA

	Normal	HF (added or not with PEG)	HF-CLA (added or not with PEG)
Body composition (%)			
Lipid	28.17±6.74^{a,b}	31.57±1.70^{a}	25.32±3.29^{b}
Protein	17.02±0.80^{a,b}	15.28±0.44^{b}	18.79±0.32^{a}
Ash	3.48±0.91^{a}	2.79±0.12^{a,b}	3.55±0.18^{a}
Water	52.89±4.95^{a}	47.18±5.71^{a,b}	53.24±7.41^{a}

Data were analyzed using one-way ANOVA followed by paired Student's *t* tests for multiple comparisons. Values (means±S.D.; *n*=10) not sharing a common superscript differ significantly (*P*<.05).

3.5. Plasma insulin or cholesterol determination

TC levels were significantly increased in mice fed the HF diet compared to mice fed the other diets, although plasma HDLC levels did not differ among the dietary groups (Table 5). In addition, the HF diet increased the plasma LDLC level by 2.2-fold when compared to the HF-CLA diet. By contrast, LDLC levels were significantly reduced in the HF-CLA dietary groups when compared to the normal or HF dietary groups. Also, mice fed HF- CLA had lower concentrations of TC when compared to those fed the normal or HF diet. The plasma insulin levels in the HF dietary groups were significantly increased, whereas HOMA levels tended to decrease in the HF-CLA-fed groups, becoming significantly different from levels observed in the HF-fed animals.

3.6. GPDH and FAS activities with HF Diet

FAS activity was significantly lower in the HF-fed groups than in the HF-CLA-fed groups (Table 6). With the HF-CLA diet, however, FAS activity was greater, and GPDH activity was lower than with the normal or HF diets.

3.7. Hepatic MDA and antioxidant defense levels

In comparison with the HF-fed animals, the MDA levels were decreased in the liver homogenates from mice fed the

Table 5
Cholesterol levels and HOMA analysis of plasma in mice fed HF diets containing CLA

	Normal	HF (added or not with PEG)	HF-CLA (added or not with PEG)
Plasma			
HDLC (mg/dl)	51.7±3.2	50.2±8.4	49.4±6.3
LDLC (mg/dl) *	63.2±1.0^{b,c}	103.7±0.2^{a}	46.8±0.7^{c}
TC (mg/dl)	138.2±11.2^{a,b}	181.3±18.9^{a}	126.2±15.4^{b}
Insulin (μIU)	11.1±0.9^{a,b}	15.7±0.8^{a}	9.2±1.3^{b}
HOMA (μU/ml) **	2.6±0.1^{b,c}	5.4±0.4^{a}	1.7±0.3^{c}
Glucose (mM)	6.6±0.4^{a,b}	7.8±0.8^{a}	4.1±0.9^{b}

Data were analyzed using one-way ANOVA followed by paired Student's *t* tests for multiple comparisons. Values (means±S.D.; *n*=10) not sharing a common superscript differ significantly (*P*<.05).

* LDLC levels were calculated by using the following Friedwald formula [16]; LDLC=TC−[(HDLC−TG)/5].

** HOMA levels were calculated by using the following Matthews formula [17]; HOMA=insulin×glucose/22.5.

Table 6
GPDH and FAS activities of adipose tissue in mice fed HF diets containing CLA

	Normal	HF (added or not with PEG)	HF-CLA (added or not with PEG)
Enzyme activity			
GPDH in AF (U/mg of protein)	1373.3±29.4[a,b]	1,534.7±39.5[a]	916.3±53.1[b]
FAS in AF (U/mg of protein)	1,710.7±63.3[a,b]	993.4±81.7[c]	1,959.9±83.1[a]

Data were analyzed using one-way ANOVA followed by paired Student's *t* tests for multiple comparisons. Values (means±S.D.; *n*=9) not sharing a common superscript differ significantly ($P<.05$).

HF-CLA diet (Fig. 1). Moreover, HF-CLA-fed mice had increased GSH levels per g liver, but not per mg of mitochondrial protein. However, no significant differences were observed among the dietary groups for SOD activity in either the liver homogenate or mitochondrial fraction (data not shown). By contrast, GPX tended to be greater in the mitochondrial fraction from HF-CLA-fed mice than from those fed the HF diet, but the GPX activity in the liver homogenate was not different between the dietary groups. Interestingly, in HF-CLA-fed animals, the GPX activity in mitochondrial fractions was significantly greater than in normal- or HF-fed animals.

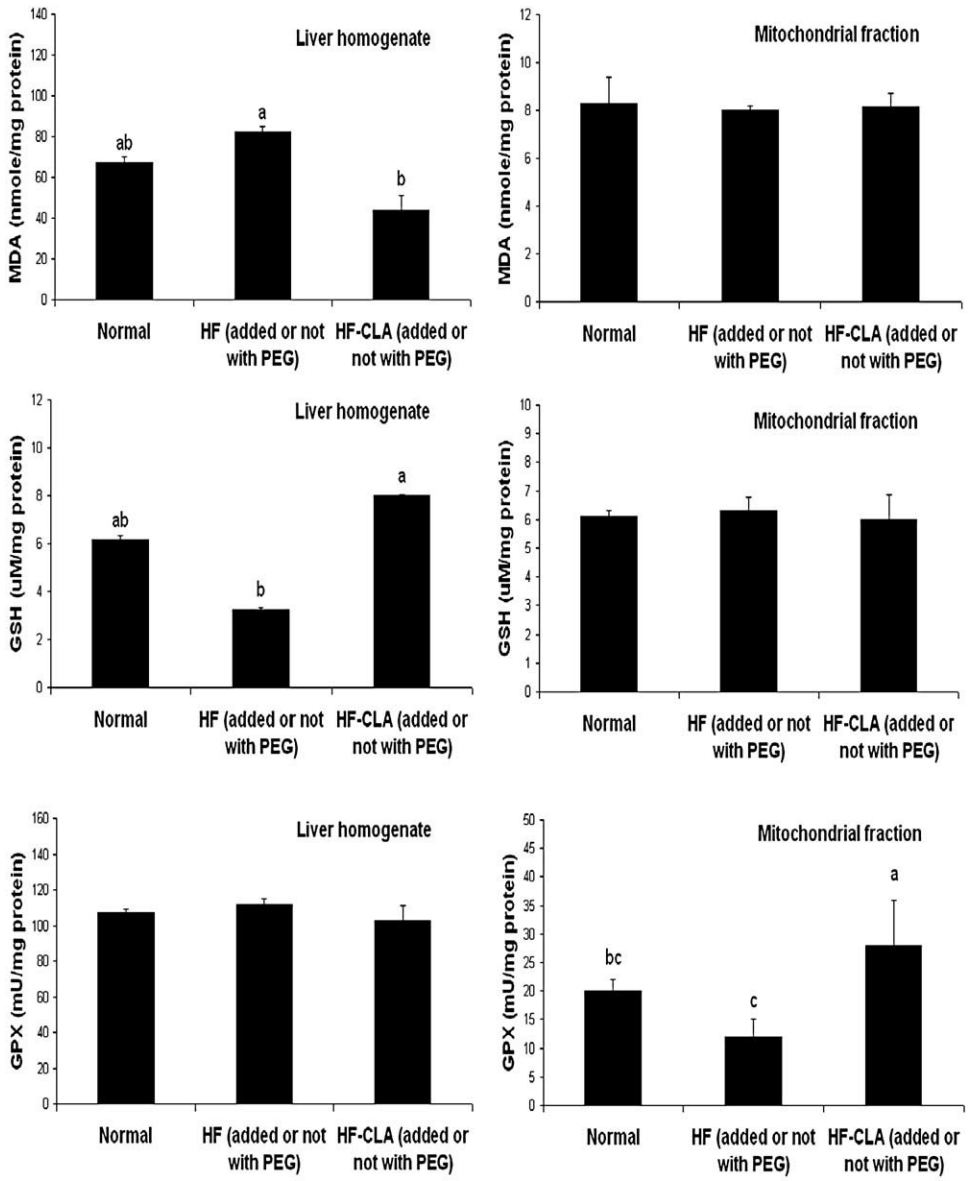

Fig. 1. Effect of PCLA diet on MDA and antioxidant defense levels in whole homogenate and mitochontrial fraction of liver. Data were analyzed using one-way ANOVA, followed by paired Student's *t* test for multiple comparisons. Values (means±S.D.; *n*=5) not sharing a common superscript differ significantly ($P<.05$).

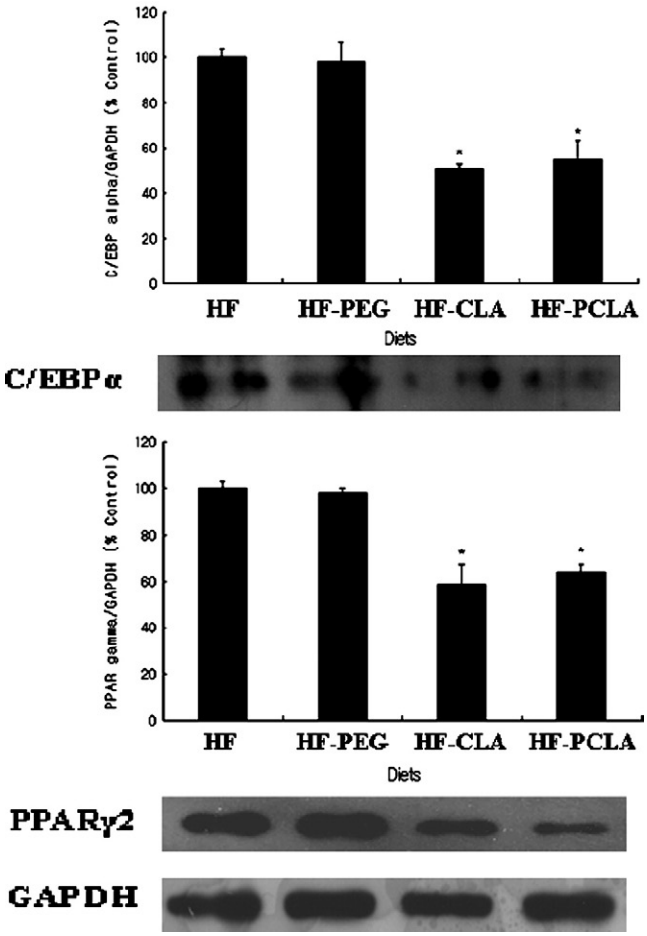

Fig. 2. Effect of PCLA diet on adipocyte marker protein expression. The primary antibodies used were mouse monoclonal PPARγ2 antibody recognizing both PPARγ isoforms and mouse monoclonal antibody against mouse C/EBPα. Secondary antibody used was horseradish peroxidase-conjugated antimouse antibody. Data are expressed as a percentage of the control level. Values are means (n=5)±S.D. *P<.05.

3.8. Adipocyte marker protein expression

Adipocyte marker protein expression (Fig. 2) was lower in HF-CLA-fed mice than in those fed the HF diet.

4. Discussion

We previously demonstrated that PEGylation increases the water solubility and enhances the bioavailability of CLA due to the biocompatible and hydrophilic properties of PEG [18]. To further investigate our previous in vitro observation, this study was designed to test the hypothesis that PCLA acts as a mediator susceptible of inducing or inhibiting specific metabolic pathways in HF diet-induced obese animals.

In this study, we investigated the combined effects of dietary PCLA on an induced prediabetic state characterized by insulin resistance and obesity in HF-fed C57BL/6J (*ob/ob*) mice [27]. Although many other investigators have reported lipoatrophia and liver steatosis in mice fed normal diets enriched with CLA, we found that a HF diet enriched in CLA or PCLA equally protect *ob/ob* mice, from liver steatosis despite a decrease in AF, which is in accordance with previous findings [28].

Our results revealed that the final body weights and weight gain in HF-fed mice were significantly greater than in mice fed a normal diet. Previous studies also supported that a HF diet increased body weight and accelerated insulin resistance [4]. Feeding HF-CLA or HF-PCLA prevented the increase in body weight that occurred with the HF diet added or not with PEG. Because of a similar food intake in both HF (with or without PEG)- and HF-CLA (or HF-PCLA)-fed mice over the whole trial, a loss of fat in feces could be expected through altered digestion and/or transit speed in CLA treated-mice [5,7]. There was apparently no marked fecal fat loss, which suggested that the absence of increased body weight with CLA was also likely due to an accelerated use of fat for body energy requirement.

Plasma insulin levels and HOMA indexes were significantly lower in mice fed the HF-CLA diets than in those fed only the HF-diets, demonstrating that CLA was a good candidate for the treatment of obesity in HF-fed C57BL/6J (*ob/ob*) mice. However, CLA used at 1.5% in the diet did not exert any particular effect in many organs of Fisher rats [29], while AKR/J mice ingesting CLA exhibited a paradoxical increase in circulating insulin levels and became insulin resistant [30] and that CLA treatments resulted in liver fat accumulation in normal C57BL/6J [31] as well as AKR/J mice [30]. All these data suggest that the biological effects of CLA depend on the animal species and the mouse strains used. Interestingly, Purushotham et al. [32] demonstrated that insulin resistance induced by CLA in male C57BL/6J mice was prevented by leptin, suggesting that the ability of CLA to increase hyperinsulinemia and hepatic steatosis in the presence of leptin may be dependent on other factors such as the basal levels of both leptin and adiponectin. Therefore, further work is needed to identify the dietary effect of CLA or PCLA on HF diet-induced obesity in various strains in the absence of leptin.

Generally, CLAs are as insoluble as FFAs [33,34]. Therefore, when given as a dietary supplement, CLA should be physically or chemically modified to enhance their water solubility and allow their clinical application [9,10]. By contrast, PEGylation increases CLA stability at pH, light and temperature when compared to the free form [11,12]. Indeed, we previously reported that PEGylation of all-transretinoic acid increases the stability of RA under light exposure with the formation of core shell-type polymeric nanoparticles by the PEGylation [35]. Also, PEG linking appears to be a useful tool to increase the water solubility of CLA [18].

The HF-CLA diet significantly reduced the carcass lipid content by reference to the HF-diet. In support of this finding, Park et al. [7] reported that CLA caused a redistribution of carcass composition, resulting in a decrease in body fat and an increase in lean tissue. In addition, the plasma level of TC is a major determinant of vascular disease

risk in human with or without symptomatic vascular diseases [36]. Usually, LDLC, the major atherogenic lipoprotein, makes up 70% of TC and contains a single apolipoprotein [37]. The present study shows that lower TC levels appear to be correlated with lower LDLC in the HF-CLA dietary groups. The increased FAS activity in abdominal fat of CLA-fed rats despite the basal HF diet could suggest a concomitant increase in lipid synthesis, but we also observed that GPDH activity that supports a key step of lipogenesis was clearly reduced. Consequently, the fatty acid synthesis pathway would be depressed in fat tissue, in accordance with findings that CLA promotes lipodystrophy through increased apoptosis [38].

Accumulation in liver cells of lipids rich in unsaturated fatty acids provides abundant substrates for lipid peroxidation in a general context of oxidative stress [39]. Lipoperoxidation may start from electron transfer-mediated reactions in peroxisomes, endoplasmic reticulum and more particularly in mitochondria through the respiratory chain activity [40]. MDA is a product of lipid peroxidation and is an index of cell oxidative stress. CLA treatment was observed to significantly decrease MDA levels in liver homogenates but not in corresponding mitochondria, which suggested that mitochondria antioxidant activities exerted their effects essentially on the extra-mitochondria domain. These effects could arise from diminished electron transferring by respiratory chain and/or from increased activities the function of which is to attenuate the levels of free radicals. The former possibility appeared unlikely because the HF-CLA group did not seem to be greatly affected by the higher dietary fat through, in addition to fecal loss, increased fatty acid oxidation rates. In contrast, the latter possibility was supported by GSH increase of liver homogenates through the increase in mitochondrial GPX activity. Indeed, GPX activity is believed to relieve cells from free radicals and to enhance the activity of the respiratory chain [41,42].

PPARγ and C/EBPα are transcription factors stimulating adipogenesis in NIH 3T3 fibroblasts [43,44] and are involved in the early stages of adipocytes differentiation [45]. Lipid accumulation and PPARγ expression were reported to be concomitantly increased on HF feeding [46], which meets the increased PPARγ and C/EBPα expression we found for AF in HF fed-mice. In contrast, in accordance with other studies [47], our results showed that CLA treatment prevented both PPARγ and C/EBPα expression, which may cause the reduction of fat tissues. We previously reported with 3T3-L1 cells that CLA caused de-differentiation of adipocytes by down-regulation of PPARγ2-induced adipogenesis [18] and might correspond to in vivo experiments showing that, through reduction of fat mass, CLA exerted beneficial effects including the attenuation of insulin resistance [5,7,26]. Similarly, feeding CLA or PCLA prevented TG accumulation both in liver and adipose tissues of C57BL/6J (*ob/ob*) mice by reduction of PPARγ and C/EBPα expression in AF.

5. Conclusions

On the whole, our data show that PEGylation increased the water solubility of CLA and thereby improved its bioavailability. The main properties of CLA were maintained with PEG-CLA, in particular, those relative to the reduction of fat accumulation by depressing lipogenic activities. As a consequence, despite the HF-diet, insulin resistance was strongly attenuated in naturally obese (*ob/ob*) mice. As CLA was used as a mixture of the *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLAs, the isomer actually responsible for the observed effects could not be identified, which needs further studies.

Acknowledgments

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