

Conjugated linoleic acid supplementation enhances insulin sensitivity and peroxisome proliferator-activated receptor gamma and glucose transporter type 4 protein expression in the skeletal muscles of rats during endurance exercise

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ABSTRACT

Objective(s): This study examined whether conjugated linoleic acid (CLA) supplementation affects insulin sensitivity and peroxisome proliferator-activated receptor gamma (PPAR- γ) and glucose transporter type 4 (GLUT-4) protein expressions in the skeletal muscles of rats during endurance exercise.

Materials and Methods: Sprague-Dawley male rats were randomly divided into HS (high-fat diet (HFD) sedentary group, n = 8), CS (1.0% CLA supplemented HFD sedentary group, n = 8), and CE (1.0% CLA supplemented HFD exercise group, n = 8). The rats in the CE swam for 60 min a day, 5 days a week for 8 weeks.

Results: The serum glucose and insulin contents and homeostasis model assessment of insulin resistance (HOMA-IR) value of the CS and CE were significantly decreased compared to those of the HS. The PPAR- γ protein expressions in the soleus muscle (SOM) and extensor digitorum longus muscle (EDL) were significantly higher in the CE than in the HS. In addition, the PPAR- γ protein expression in the SOM of the CS was significantly higher than that in the HS. On the other hand, the GLUT-4 protein expression of the SOM in the CE was significantly higher compared to that in the HS. However, there was no significant difference in GLUT-4 protein expression in the EDL among the groups.

Conclusion: CLA supplementation with/without endurance exercise has role in improvement of insulin sensitivity. Moreover, when CLA supplementation was accompanied by endurance exercise, the PPAR- γ protein expression in SOM and EDL and the GLUT-4 protein expression in SOM were enhanced compared with the control group.

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Introduction

Conjugated linoleic acid (CLA) is a fatty acid mixture of positional geometric isomers of octadecadienoic acid (linoleic acid 18: 2n-6) with a conjugated double-bond system (1). Commercially, mixed isomer CLA is marketed as a weight-loss supplement. Different isomers of CLA have varied biological functions, such as reducing carcinogenesis, decreasing adipose mass, and modulating immune function and type 2 diabetes (2). CLA also induces hyperinsulinemia and insulin resistance, primarily in mice (3). However, the effects of CLA supplementation on skeletal muscle are still unclear (4).

Obesity is becoming a major public health problem

in affluent societies (5). It is well known that diet control and physical exercise are the two main approaches to suppress obesity (6, 7). Previous studies reported that a HFD increases the total energy intake, and that excess dietary fat is greater stored than excess dietary carbohydrate or protein. Thus, an increase in excessive energy intake from fat can reduce physical activity, and this decline of physical activity causes obesity (8, 9).

Physical activity has been considered as a cornerstone in the treatment of obesity (10, 11). Among various types of physical activities, endurance exercise has long been reported to reduce body weight, ameliorate postprandial triglyceride

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Table 1. Composition of experimental diets

Variable	HFD ¹⁾	CLA Suppl. diet ²⁾
Casein	0	200.0
Starch	200.0	200.0
Sucrose	150.0	150.0
Lard	350.0	337.0
Cellulose	50.0	50.0
Mineral mix	35.0	35.0
Vitamin mix	10.0	10.0
DL-methionine	3.0	3.0
Choline barbiturate	2.0	2.0
DL-a-tocopherol	1.2	1.2
c-9, t-11 CLA3)	-	6.5
t-10, c-12 CLA3	-	6.5
Energy (kcal/kg)	5350.0	5350.0
Protein (% kcal/g)	15.0	15.0
Carbohydrate (% kcal/g)	26.0	26.0
Fat (% kcal/g)	59.0	59.0

1) 35% fat of total diet weight were supplied for the HS group 2) 1.0% CLA was added and adjusted to the high-fat diet (HFD) for the CS and CE group

3) 76.81% CLA mixture

CLA: conjugated linoleic acid, CS: CLA supplemented HFD sedentary, CE: CLA supplemented HFD exercise, HS: HFD sedentary

response, increase the rate of fat oxidation, and improve insulin sensitivity (12-15). However, the physiological and molecular mechanisms for these benefits have not been completely understood (16).

Glucose transporter-4 (GLUT-4) translocation to the plasma membrane through the insulin signaling pathway is well proven (17,18). In addition, thiazolidinediones, which are peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists that decrease insulin resistance, are widely used as a treatment for patients with type 2 diabetes (19). Although PPAR- γ is highly expressed in adipose tissue than in muscle, muscle specific-PPAR- γ depletion is susceptible to developing insulin resistance in mice (20). Previous studies reported that exercise had improved insulin sensitivity due to an increase in PPAR- γ protein expression (20-21).

The aim of this study was to investigate the effects of CLA supplementation on the insulin resistance and PPAR- γ and GLUT-4 protein expression in the skeletal muscles of rats on a HFD during endurance exercise.

Materials and Methods

Experimental animals and diet

Six-week-old male Sprague-Dawley rats weighing 230 to 250 g were obtained (Samtako Co., Osan,

Korea) and individually housed in a controlled environment at 23±1 °C with 50±5% relative humidity, under a 12-hr light-dark cycle. All animals were given free access to tap water and food. After an acclimatization period of 1 week, all rats were randomly divided into 3 groups: HS, HFD (35% fat of total diet weight) (22) sedentary group; CS, CLA supplemented HFD sedentary group; and CE, CLA supplemented HFD exercise group. For CLA supplementation, 1.0% CLA (76.82% CLA mixture; 36.8% *cis*-9,*trans*-11 CLA, 37.8% *trans*-10, *cis*-12 CLA, and 1.2% *trans*-9,*trans*-11 CLA) (HK Biotech Co., Gyeongnam, Korea) was substituted for dietary fat in the adjusted HFD (Table 1). During this period, dietary intake was measured daily, and the change in the body weight of each animal was noted weekly. The dietary regimens were based on AIN-76 of the animal diet and were modified from a previous study (23). All of the experimental protocols were approved by the Animal Study Committee of Sunmoon University.

Exercise protocol and sample collection

The exercised rats swam for 60 min a day, 5 days a week for 8 weeks. The water temperature of the swimming pool was maintained at approximately 35±1 °C in a plastic barrel (depth; 50 cm, radius; 25 cm). At the end of the experimental protocol, in an overnight-fasted state, the rats were sacrificed by exsanguination, and blood was drawn from the left ventricle under light diethyl ether anesthesia after the 12-hr fasting period. The skeletal muscles: soleus muscles (SOM) as slow muscle fiber and extensor digitorum longus muscle (EDL) as fast muscle fiber (24) and abdominal fat (AFT) as fat tissue were dissected and immediately snap-frozen in liquid nitrogen. The skeletal muscles and fat tissue were stored at -70 °C until they were analyzed.

Biochemical assays

The serum glucose, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL) levels were analyzed using commercial enzymatic kits (Asan Pharmaceutical Co., Yongin, Korea). The fasting insulin level was measured using standard radio-immunity kits (Linco Research, Inc., St. Louis, MO, USA). The serum low-density lipoprotein cholesterol (LDL) level was calculated from TG, TC, and HDL concentrations using the following Friedwald formula: {TC - (HDL + (TG/5))}. Insulin resistance was calculated according to the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: {fasting glucose level (mmol/l) × fasting insulin level (uU/ml)}/22.5.

Western blot analysis

In order to analyses PPAR- γ and GLUT-4 protein expression, muscles (the SOM and EDL) were

Table 2. The level of body weight, weight gain, fat weight, and skeletal muscles weight among groups

Variables (g)	HS (n = 8)	CS (n = 8)	CE (n = 8)
Body weight	468.1 ± 3.3	433.2 ± 5.9***	414.2 ± 6.5***, #
Weight gain	193.7 ± 3.2	167.7 ± 3.1***	149.7 ± 5.5***, ##
AFT	17.5 ± 0.6	12.6 ± 0.5***	10.3 ± 0.8***, #
SOM	0.125 ± 0.003	0.159 ± 0.004***	0.162 ± 0.007***
EDL	0.167 ± 0.004	0.172 ± 0.006	0.178 ± 0.002

homogenized on ice with a polytron homogenizer in 20 mmol/l Tris-HCl buffer (pH 7.5) containing 5 mmol/l ethylenediaminetetraacetic acid, 2 mmol/l phenylmethylsulfonyl fluoride, and 1:200 protease inhibitor cocktail (Sigma, St Louis, MO, USA). The protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard. An aliquot of tissue extract containing 20 µg of protein was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. After electrophoresis, the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA) in a semi-dry blotting apparatus (Bio-Rad, Hercules, CA, USA). After treating with blocking buffer (phosphate-buffered saline (PBS) containing 10% skim milk) for 90 min, the membrane was incubated with primary polyclonal antibodies for 2 hr, followed by five 10-min washes with PBS (5% tween 20). The membranes were washed and then incubated with horseradish peroxidase (HRP)-conjugated anti-goat immunoglobulin G (IgG) or anti-rabbit IgG (Santa Cruz, CA, USA) for 1 hr, followed by five 10-min washes with PBS (5% Tween 20). The target proteins were detected using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The films were photographed and the protein bands of interest were quantified with band analyzer software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The data were expressed as the means ± standard error of the mean (SEM) using the SPSS/PC program statistical analyses were conducted using one-way

ANOVA followed by LSD's *post hoc* test to verify significant difference among groups. Statistical significance was set at $P < 0.05$.

Results

The weight-related variables

The changes in the weight-related variables are summarized in Table 2. The body weight, weight gain, and AFT in the CS and CE were significantly lower than those in the HS ($P < 0.001$). Additionally, the body weight ($P < 0.05$), weight gain ($P < 0.01$), and AFT mass ($P < 0.05$) in the CE were significantly reduced compared to those in the CS. In the weight of the skeletal muscles, the SOM in the CS and CE were significantly increased compared to those in the HS ($P < 0.001$), whereas the EDL did not show any significant differences among the groups.

Changes in serum parameters

The changes in the serum parameters are presented in Table 3. The serum TG, TC, HDLC, and LDLC levels in the CS and CE were significantly lower than those in the HS ($P < 0.001$). Furthermore, the serum TC concentration was found to have significantly reduced in the CE compared to that in the CS ($P < 0.01$).

Changes in serum glucose and insulin contents and HOMA-IR level

As shown in Figure 1, the serum glucose and insulin contents and HOMA-IR value of the CS (glucose: $P = 0.001$, insulin: $P = 0.006$, HOMA-IR: $P = 0.004$) and CE (glucose: $P = 0.001$, insulin: $P = 0.001$, HOMA-IR: $P = 0.001$) were significantly decreased

Table 3. The level of serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and triglycerides among groups

Variables (g)	HS (n = 8)	CS (n = 8)	CE (n = 8)
TC	74.6 ± 2.5	67.6 ± 2.9***	62.4 ± 1.6***, ##
HDLC	20.1 ± 1.3	33.0 ± 2.5***	37.2 ± 3.3***
LDLC	26.0 ± 1.2	14.4 ± 2.8***	7.7 ± 3.3***
TG	125.9 ± 3.8	104.6 ± 2.6***	99.6 ± 1.1***

Data are presented as the mean ± SEM. *** $P < 0.001$ compared with the HS group; ## $P < 0.01$ compared with the CS group. TG; triglycerides, TC; total cholesterol, HDLC; high density lipoprotein cholesterol, LDLC; low density lipoprotein cholesterol. CLA; conjugated linoleic acid, CLA; conjugated linoleic acid, CS; CLA supplemented HFD sedentary, CE; CLA supplemented HFD exercise, HS; HFD sedentary

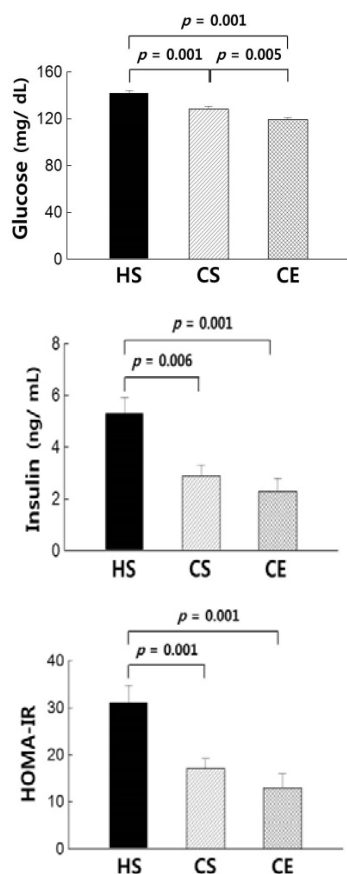


Figure 1. Changes of serum glucose and insulin contents and homeostasis model assessment of insulin resistance (HOMA-IR) level among groups

compared to those of the HS. Furthermore, the glucose content of the CE was significantly reduced compared to that of the CS ($P=0.005$)

PPAR- γ and GLUT-4 protein expression level according to skeletal muscle fiber type

As shown in Figure 2, PPAR- γ protein expressions in the SOM ($P=0.005$) and EDL ($P=0.043$) were higher in the CE than those in the HS. In addition, the PPAR- γ protein expression in the SOM of the CS ($P=0.035$) was significantly higher than that in the HS. On the other hand, the GLUT-4 protein expression of the SOM in the CE was significantly higher compared to that in the HS ($P=0.034$). However, there was no significant difference in the GLUT-4 expression of the EDL among the groups (Figure 3).

Discussion

To the best of our knowledge, this is the first study to investigate the effects of CLA supplementation with/without endurance exercise on insulin resistance and PPAR- γ and GLUT-4

protein expression by analyzing the skeletal muscle fiber types of rats on a HFD. Our results suggest that CLA supplementation and endurance exercise independently affected the insulin sensitivity and PPAR- γ and GLUT-4 protein expression according to the skeletal muscle fiber type.

Previous studies have reported that CLA decreases body mass and fat mass, while improving serum lipids profiles (25-28). Our results show that not only weight-related parameters such as body weight, weight gain, and fat tissue weight, but also serum parameters such as TG, TC, HDLC, and LDLC in the CLA-supplemented groups with/without endurance training were significantly reduced compared to the control group. Some studies reported that endurance exercise results in the significant decrease of fat mass and body mass as well as an improved serum lipids profiles (29, 30). However, in our study, in the case of CLA supplementation, the endurance exercise group have tended lower in body weight, weight gain, AFT mass, and glucose and TC concentrations compared to the CS. These results suggest that endurance exercise during CLA supplementation might result in the utilization of fat as a substrate during exercise, and that endurance exercise reduces body mass and body fat mass. Previous studies reported that the lipid profile values did not increase above the normal range in six-week-old male Sprague-Dawley rats fed with

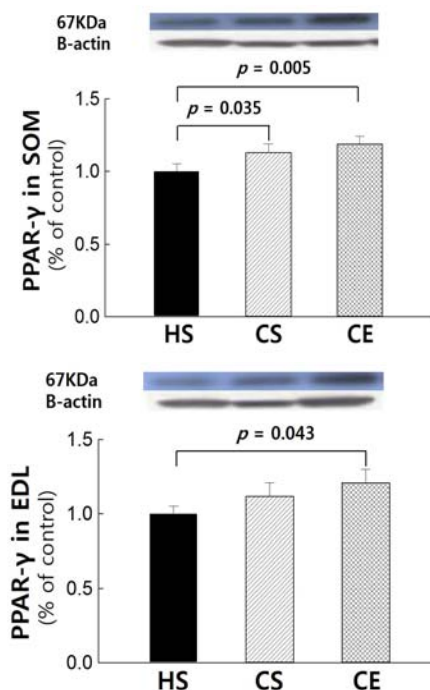


Figure 2. Changes of peroxisome proliferator-activated receptor gamma (PPAR- γ) protein expression level in skeletal muscles. SOM; soleus muscle, EDL; extensor digitorum longus muscle

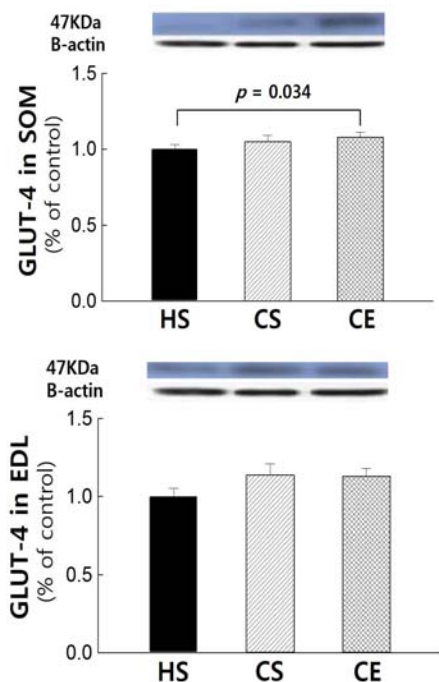


Figure 3. Changes of glucose transporter type 4 (GLUT-4) protein expression level in skeletal muscles. SOM; soleus muscle, EDL; extensor digitorum longus muscle

HFD for 8 weeks (20, 23). However, in our study, statistically significant differences were shown in the serum lipid profile concentrations after 8 weeks of treatment. Those levels may ameliorate serum lipid profiles when one engages in at least 8 weeks of endurance exercise with/without CLA supplementation. These results suggest that the combination of CLA supplementation and endurance exercise may be recommended for utilizing fat as a substrate, reducing body mass and body fat mass, and ameliorating serum lipid profiles compared to CLA supplementation or endurance exercise alone rather than in conjunction with each other.

Many animal studies have tried to induce a model of obesity using a dietary composition of approximately 35 to 40% lard and also shown that HFDs induce hyperglycemia, hyperinsulinemia and insulin resistance (31-34). It is also reported that endurance exercise ameliorates the serum insulin concentration and HOMA-IR level in rats fed a HFD (35). In this study, CLA supplementation with/without endurance exercise decreased the insulin content and HOMA-IR value. These results suggested that CLA supplementation could potentially results in lower insulin resistance regardless of participation in endurance exercise.

The effects of CLA on skeletal muscle are still unclear. The most pronounced effect of CLA isomer feeding was a stimulation of protein synthesis, which enhanced lean body mass. This stimulatory effect

was mainly observed in the muscles of sedentary rats (36-39). Regular physical activity leads to a number of adaptations in skeletal muscle (40). Skeletal muscle fiber types have been traditionally classified as "slow twitch" and "fast twitch" according to their expression of myosin heavy chain isomers. The slow twitch fibers mainly depend on oxidative pathways, and the fast twitch fibers mainly depend on glycolytic pathways for ATP production (41). In this study, the SOM and EDL, which are hind-limb muscles, were selected as typical slow and fast twitch fibers, respectively. We found a considerably increased SOM mass in the case of CLA supplementation regardless of participation in endurance exercise, while there was no significant difference in EDL mass. This result seems to indicate that the increase in SOM mass is a result of swimming exercise.

It is well established that the mechanism for elevating glucose uptake is mainly by the translocation of GLUT-4 vesicles from the intracellular pool to the plasma membrane through insulin binding to its receptor (42-44). Although the exact correlation between PPARs and GLUT-4 protein expression is not clearly understood, PPAR- γ protein is reported to play an important role for regulation of GLUT-4 gene expression in skeletal muscle tissues (45). Our study also showed that the PPAR- γ protein expression in the SOM and EDL of the CE was significantly higher compared to that in the HS, and the PPAR- γ protein expression in the SOM of the CS tended to be significantly higher than that in the HS. Previous studies have shown that PPAR- γ protein expression increases with exercise in plasma and adipose tissue (46, 47), and is upregulated by endurance or a moderate level of intense exercise (48, 49). Our finding is in accordance with previous studies, which show that the PPAR- γ protein expression of the SOM in the CE rats was significantly higher than that of the HS rats. However, the PPAR- γ protein expression of the CLA-supplemented non-exercised group in the EDL did not show significant improvement compared to the HS group. As mentioned above, the soleus muscle, as slow twitch fibers, were mainly activated during swimming, and it might be assumed that changes in protein expression are affected by external factors such as obtained muscle type during exercise and the type and duration of the exercise. Thus, further studies considering the time and type of exercise are needed to better elucidate the relationship between exercise and protein expression.

Our study showed that the muscle GLUT-4 protein expression of the SOM in the CE was significantly higher than that in the HS, while the GLUT-4 protein expression of the SOM in the CS was not significantly different compared to that in the HS.

It is reported that the slow twitch fibers are more responsive to insulin than the fast twitch fibers (50). These results are likely due to the fact that swimming exercise mainly mobilizes the SOM, which increases in response to endurance training, thus facilitating glucose uptake into the trained muscle. In our study, the GLUT-4 protein expression in the EDL did not show significant improvement. Taken together, our results indicate that the combined case of CLA supplementation and endurance exercise improve not only the GLUT-4 expression level, but also the PPAR- γ expression level in the SOM. Skeletal muscle comprises a relatively large mass in the body and is an important target tissue for glucose metabolism by insulin (51, 52). Therefore, we can assume that the significant changes in the GLUT-4 activity of the CE in the SOM may have significant physiological effects because the soleus muscle is the major target tissue of insulin for glucose uptake during swimming.

Taken together, even when accompanied by a HFD, CLA supplementation with/without endurance exercise plays a role in the suppression of obesity and the improvement of insulin sensitivity. When CLA supplementation was accompanied by endurance exercise, the PPAR- γ protein expression in the SOM and EDL and the GLUT-4 protein expression in the SOM were enhanced compared to the control group. In addition, the PPAR- γ protein expression in the SOM of the CS was enhanced by only CLA supplementation.

Conclusion

In conclusion, CLA supplementation under a non-endurance exercising condition in rats may play a role in enhancing the PPAR- γ protein expression in red muscles. In addition, CLA supplementation under an endurance exercise condition in rats may enhance the PPAR- γ protein expression in the SOM and EDL and the GLUT-4 protein expression in the SOM. However, there was no synergic effect of CLA supplementation and endurance exercise on insulin resistance in the serum and protein expression in skeletal muscles of rats under the condition of CLA supplementation with/without endurance exercise.

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Conflict of interests

Authors have no conflict of interests.

References

1. Charlotte AM. Muscle development and obesity. *Organogenesis* 2008; 4:158-169.
2. David S. Exercise, appetite and appetite-regulation hormones: implications for food intake and weight control. *Nutri Metab* 2010; 57:36-42.
3. Feinle-Bisset C. Modulation of hunger and satiety: hormones and diet. *Curr Opin Clin Nutr Metab Care* 2014; 17:458-64.
4. Eu CH, Lim WY, Ton SH, bin Abdul Kadir K. Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet induced obese rats. *Lipids Health Dis* 2010; 9:81-89.
5. Patric S, Klaas RW. The role of high-fat diet and physical activity in the regulation of body weight. *Br J Nutr* 2000; 84:417-427.
6. Pescatello LS, VanHeest JL. Physical activity mediates a healthier body weight in the presence of obesity. *Br J Sports Med* 2000; 34:86-93.
7. Goh VH, Hart WG. Associations of physical exercise as a lifestyle habit with lean and fat body mass and handgrip strength and age in Asian men. *Ageing Male* 2014; 17:131-135.
8. Calegari VC, Zoppi CC, Rezende LF, Siverira LR, Cameiro EM, Boschero AC. Endurance training activates AMP-activated protein kinase, increases expression of uncoupling protein 2 and reduces insulin secretion from rat pancreatic islets. *J Endocrinol* 2011; 208:257-264.
9. Hung YH, Linden MA, Gordon A, Rector RS, Buhman KK. Endurance exercise training programs intestinal lipid metabolism in a rat model of obesity and type 2 diabetes. *Physiol Rep* 2015; 3:e12232.
10. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 1998; 49:235-261.
11. Bente K. Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev* 2006; 86:205-243.
12. Gonçalves DC, Lira FS, Carnevali LC Jr, Rosa JC, Pimentel GD, Seelaender M. Conjugated linoleic acid: good or bad nutrient. *Diabetol Metab Syndr* 2010; 2:62-73.
13. Moon HS, Lee HG, Seo JH, Chung CS, Kim TG, Choi YJ, et al. 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. *J Nutr Biochem* 2009; 20:187-194.
14. Azain MJ, Hausman DB, Sisk MB, Flatt WP, Jewell DE. Dietary conjugated linoleic acid reduces fat adipose tissue cell size rather than cell number. *J Nutr* 2000; 130:1548-1554.
15. Angela AW, Aparna P, Li-Fen L, Marths AB. Conjugated linoleic acid fails to worsen insulin resistance but induces hepatic steatosis in the presence of leptin in ob/ob mice. *J Lipid Res* 2008; 49:98-106.
16. Jhao J, Tian Y, Xu J, Liu D, Wang X, Zhao B. Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. *Lipid Health Dis* 2011; 10:225-231.
17. Brozinick JT, Etgen GJ, Yaspelkis BB, Kang HY, Ivy JL. Effects of exercise training on muscle GLUT-4 protein content and translocation on obese Zucker rats. *Am J Physiol Endocrinol Metab* 1993; 265:E419-E427.

18. Dohm GL. Exercise effects on muscle insulin signaling and action invited review; Regulation of skeletal muscle GLUT-4 expression by exercise. *J Appl Physiol* 2002; 93: 782-787.
19. Hammarstedt A, Anderson CX, Rotter SV, Smith U. The effects of PPAR gamma ligand on the adipose tissue in insulin resistance. *Prostaglandins Leukot Essent Fatty Acids* 2005; 73:65-75.
20. Kang J, Lee J, Kwon D, Song Y. Effect of *Opuntia humifusa* supplementation and acute exercise on insulin sensitivity and associations with PPAR- α and PGC-1 α protein expression in skeletal muscle of rats. *Int J Mol Sci* 2013; 4:7140-7154.
21. Russell AP, Feilchenfeldt J, Schreiber S, Praz M, Crettenand A, Gobelet C, et al. Endurance training in humans leads to fiber type specific increases in levels of peroxisome proliferator activated receptor gamma coactivator 1 and peroxisome proliferator activated receptor alpha in skeletal muscle. *Diabetes* 2003; 52: 2874-2881.
22. Hariri N, Thibault L. High-fat diet induced obesity in animal models. *Nutr Res Rev* 2010; 23:270-299.
23. Kwon DK, Hwang KH, Kim YK, Lee KH, Song YJ. Effects of swimming exercise and soybean supplementation on the immune functions of rats fed a high fat diet. *Clin Exp Pharmacol Physiol* 2008; 35:638-642.
24. Armstrong RB, Phelps RO. Muscle fiber type composition of the rat hind limb. *Am J Anat* 1984; 171:259-272.
25. DeLany JP, Blohm F, Truett AA, Scimeca J, West DB. Conjugated linoleic acid reduces body fat content in mice without affecting energy intake. *Am J Physiol* 1999; 276:R1172-R1177.
26. Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 1997; 32:853-858.
27. Koba K, Akahoshi A, Yamasaki M, Tanaka K, Yanada K, Iwata T, et al. Dietary conjugated linoleic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. *Lipids* 2002; 37:343-350.
28. Poulos SO, Sisk M, Hausman DB, Azain MJ, Hausman GJ. Pre- and postnatal dietary conjugated linoleic acid alters adipose development, body weight gain and body composition in Sprague-Dawley rats. *J Nutr* 2001; 131:2722-2731.
29. Kim CH, Youn JH, Park JY, Hong SK, Park KS, Park SW, et al. Effects of high-fat diet and exercise training on intracellular glucose metabolism in rats. *Am J Physiol Endocrinol Metab* 2000; 278:977-984.
30. Costa-Júnior JM, Ferreira SM, Protzek AO, Santos GJ, Cappelli AP, Silveira LR, et al. Endurance training inhibits insulin clearance and IDE expression in Swiss mice. *PLoS One* 2015; 10:e0118809.
31. Yamasaki B, Ikeda A, Oji M, Tanaka Y, Hirao A, Kasai M, et al. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague Dawley rats fed various fat-level diets. *Nutrition* 2003; 19:30-35.
32. Hariri N, Thibault L. High-fat diet induced obesity in animal models. *Nutr Res Rev* 2010; 23:270-299.
33. Eu CH, Lim WY, Ton SH, bin Abdul Kadir K. Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet induced obese rats. *Lipids Health Dis* 2010; 9:81-89.
34. Straczkowski M, Kowaiska I, Dzienis-Straczkowska S, Kinalski M, Górski J, Kinalska L. The effect of exercise training on glucose tolerance and skeletal muscle triacylglycerol content in rats fed with a high-fat diet. *Diabetes Metab* 2001; 27:19-23.
35. Haghshenas R, Jafari M, Ravasi A, Kordi M, Gilani N, Shariatzadeh M, et al. The effect of eight weeks endurance training and high-fat diet on appetite-regulating hormones in rat plasma. *Iran J Basic Med Sci* 2014; 17:237-243.
36. Tarnopolsky M, Zimmer A, Paikin J, Safdar A, Aboud A, Pearce E, et al. Creatine monohydrate and conjugated linoleic acid improve strength and body composition following resistance exercise in older adults. *PLoS One* 2007; 2:e99120.
37. Patureau Mirand P, Mosoni L, Arnal-Bagnard MA, Faulconnier Y, Chardigny JM, Chilliard Y. Dietary conjugated linoleic acid has limited effects on tissue protein anabolism in sedentary and exercising adult rats. *Reprod Nutr Dev* 2006; 46:621-632.
38. Livisay SA, Zhou S, Ip C, Decker EA. Impact of dietary conjugated linoleic acid on the oxidative stability of rat liver microsomes and skeletal muscle homogenates. *J Agric Food Chem* 2000; 48:4162-4167.
39. Park Y, Albright KJ, Storkson JM, Liu W, Cook ME, Pariza MW. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids* 1999; 34:243-248.
40. Röckl KS, Witczak CA, Goodyear LJ. Signaling mechanisms in skeletal muscle; acute responses and chronic adaptations to exercise. *IUBMB Life* 2008; 60:145-153.
41. Yoshika Y, Masuda T, Nakano H, Miura H, Nakaya S, Itazawa S, et al. *In vitro* IH-NMR spectroscopic analysis of metabolites in fast- and slow-twitch muscles of young rats. *Magn Reson Med Sci* 2002; 1:7-13.
42. Vyas D, Kadegowda AKG, Erdman RA. Dietary conjugated linoleic acid hepatic steatosis: Species-specific effects on liver and adipose lipid metabolism and gene expression. *J Nutr Metab* 2012; 2012:932928-932940.
43. Kern M, Wells JA, Stephens JM, Elton CW, Friedman JE, Tapscott EB, et al. Insulin responsiveness in skeletal muscle is determined by glucose transporter protein level. *Biochem J* 1990; 270:397-400.
44. Friedman JE, Sherman WM, Reed MJ, Elton CW, Dohm GL. Exercise training increases glucose transporter protein GLUT-4 in skeletal muscle of obese Zucker rats. *FEBS Lett* 1990; 268:13-16.
45. Kumar PM, Venkataranganna MV, Manjunath K, Viswanatha GL, Ashok G. Methanolic extract of *Momordica cymbalaria* enhances glucose uptake in L6 myotubes *in vitro* by up-regulating PPAR- γ and GLUT-4. *Chin J Nat Med* 2014; 12:895-900.
46. Li M, Bai Y, Chen C, Cui J, Xu X, Dai Y. Effects of exercise and conjugated linoleic acid on PPAR γ in adolescent obese rats. *Wei Sheng Yan Jiu* 2015; 44:179-184.
47. Li M, Bai Y, Jianfei C, Xiaodong X, Yuanyuan D, Jing Z. Effects of different exercise intensity on PPAR γ and

- relative index in adolescent obesity rats. Wei Sheng Yan Jiu 2014; 43:732-737.
48. Phillippe PM, Laurent M, Marie-Agnes AB, Yannick F, Jean-Michel C, Yves C. Dietary conjugated linoleic acid has limited effects on tissue protein anabolism in sedentary and exercising adult rats. *Reprod Nutr Dev* 2006; 46:621-632.
49. Lira FS, Yamahita AS, Uchida MC, Zanchi NE, Guaiano B, Martins JE, *et al*. Low and moderate rather than high intensity strength exercise induces benefit regarding plasma lipid profile. *Diabetol Metab Syndr* 2010; 2:31-36.
50. Lillioja S, Young AA, Culter CL. Skeletal muscle capillary density and fiber type are possible determinants of *in vivo* insulin resistance in man. *J Clin Invest* 1987; 80:415-424.
51. Henriksson J. Effect of training and nutrition on the development of skeletal muscle. *J Sports Sci* 1995; 13:S25-30.
52. Kolka CM, Richey JM, Castro AV, Broussard JL, Ionut V, Bergman RN. Lipid-induced insulin resistance does not impair insulin access to skeletal muscle. *Am J Physiol Endocrinol Metab* 2015; [Epub ahead of print].

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