Effects of *Amomum cadamomum* Linne Extract on TNF-α-induced Inflammation and Insulin Resistance in 3T3-L1 Adipocytes

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Amomum cadamomum Linne (ACL) has long been utilized against the inhibited qi movement related diseases such as dyspepsia, acute gastroenteritis, vomiting and diarrhea in Korean medicine. We speculated that ACL could improve the metabolic disorders such as obesity and type 2 diabetes through removing the phlegm-dampness and promoting the qi movement or stagnation. This study was designed to investigate effects and molecular mechanisms of ACL extract on the improvement of adipocyte dysfunction induced by TNF-α in 3T3-L1 adipocytes. Potential roles of ACL extract in the lipogenesis, inhibition of inflammatory cytokines and insulin resistance, were investigated in this study. Also, we examined the adipose genes and signaling molecules related to insulin resistance and glucose uptake to elucidate its mechanism. Our data demonstrated that TNF-α significantly incresed the release of lipid droplets and the production of MCP-1 and IL-6 from adipocytes. In gene expression, TNF-α reduced the expression of aP2, PPARγ, C/EBPα, GLUT4, and IRS-1 related to lipogenesis and insulin sesitivity, while TNF-α increased the expression of MCP-1 related to inflammation. In addition, TNF-α down-regulated the PPARγ and IRS-1 protein and up-regulated the IRS-1 Ser307 phosphorylation. These alterations induced by TNF-α were prevented by the treatment of ACL extract. Thus, our results indicate that ACL extract can be used to prevent from the TNF-α-induced adipocyte dysfunction through insulin and PPARγ pathways.

keywords : *Amomum cadamomum* Linne, TNF-α, Inflammation, Insulin resistance

Introduction

Insulin resistance is one of the central factors in the pathogenesis of methabolic syndromes such as, diabetes, cardiometabolic syndromes, and obesity[1,2]. Obesity linked insulin resistance is associated with chronic low grade inflammation in adipocytes. TNF-α is the first one to connect obesity, inflammation and insulin resistance[3,4]. The over-expression of TNF-α in adipocytes triggers the release of other proinflammatory cytokines and contributes to the progression of insulin resistance[5,6]. *Amomum cadamomum* Linne (ACL) has long been utilized against the inhibited qi movement related diseases such as dyspepsia, acute gastroenteritis, vomiting and diarrhea in Korean medicine. It has reported that ACL as composition of multi-herbal extract has anti-diabetic effects[7] and ACL has platelet protection effects against aggregation and lipid peroxidation[8]. However, it remains less clear whether ACL plays a role in adipose dysfunction related to insulin resistance. The 3T3-L1 cell line has been widely used as model for processes in mature adipocytes in biological research on adipose tissue[9]. The adipocyte is not only a lipid depository but a key metabolic regulator responsible for the production of cytokines, metabolic substrates, and adipokines, wielding influence over metabolism both locally and on a systemic level. Therefore, previous research has concentrated on the deregulation of metabolism within adipose tissue that may contribute to the wider effects of type 2 diabetes mellitus, the metabolic syndrome, and obesity[10-12].

Thus, this study was designed to investigate effects and molecular mechanisms of ACL on the improvement of adipocyte dysfunction induced by TNF-α in 3T3-L1 adipocytes. Our findings indicate that ACL extract could improve the TNF-α-induced inflammation and insulin resistance in 3T3-L1 adipocytes.

Materials and Methods

1. Materials

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Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine calf serum (BCS), penicillin and streptomycin mixture were obtained from Gibco (Grand Island, NY, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), and insulin were purchased from Sigma Aldrich (St Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits for cytokines were purchased from BD Biosciences Pharmingen (SanDiego, CA, USA). Phospho-specific IRS-1 Ser307 and total antibodies to IRS-1, PPARγ, and β-actin were obtained from Cell Signaling Technologies (Beverly, MA, USA). All other reagents were of the highest grade commercially available.

2. Amomum cadamomum Linne extracts (ACL) Preparation

ACL was isolated from the Amomum cadamomum Linne purchased from Omniherb (Daegu, Gyeongbuk, Korea). Amomum cadamomum Linne of 100 g was extracted with 1.5 L of water at 100℃ for 3 h. The extract was filtered and evaporated on a rotary evaporator (EYELA, Tokyo, Japan) under a reduced pressure. The extract was lyophilized and the yield of the extract was approximately 1.8%. A voucher specimen (DKMP-201507-ACL) was deposited at Korean Medical Physiology Laboratory, Dongeui University. The extract power was stored at −20℃ until use.

3. Cell culture

Mouse 3T3-L1 preadipocytes (CL-173) obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were grown in DMEM supplemented with 10% BCS, 100 U/mL of penicillin and 100 μg/mL of streptomycin under a humidified atmosphere with 5% CO_2 at 37℃. For induction of differentiation of 3T3-L1 preadipocytes, 2 day post confluent cells were incubated in differentiation medium containing 0.5 mM IBMX, 1 μg/mL insulin and 1 μM DEX in DMEM containing 10% FBS. After 2-3 days, the cell culture medium was changed to DMEM containing 1 μg/mL insulin and 10% FBS (maintenance medium). The medium was replaced with fresh maintenance medium every 2 days. Adipocytes were used for experiments on 8-9th day of the initiation of differentiation.

4. MTT assay

Cell viability was estimated by the MTT assay. 3T3-L1 preadipocytes were incubated into 24-well plate (5×10^4 cells/mL) and cultured overnight. After incubation, the culture medium was replaced with complete growth medium and the cells were either left untreated or treated with ACL (0.01, 0.1, 1, 10, 100, and 500 μg/mL) for 24 h at 37℃ in 5% CO_2. MTT (5 mg/mL) was added to each well and the plates were incubated at 37℃ in the dark for 4 h. The supernatant of each well was vacuum-aspirated and 500 μl of dimethylsulfoxide (DMSO) was added to each well. The plates were agitated to enhance the dissolution of the formazan that formed. Cell viability was determined using a Spectra Max M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 540 nm.

5. Adipocyte dysfunction induction and ACL treatment

Insulin resistance was induced in 3T3-L1 adipocytes by recombinant mouse TNF-α (R&D systems, Minneapolis, Minnesota, USA) treatment. Fully differentiated 3T3-L1 adipocytes were treated with 10 ng/mL TNF-α for 24 h. All the parameters were measured after 24 h of incubation of cells with TNF-α in presence or absence of 100 μg/ml of ACL.

6. Oil red O staining

3T3-L1 cells were washed with 1× phosphate-buffered saline (PBS) and fixed with 10% formalin-PBS solution for 1 h. After removing this solution, the differentiated cells were stained with Oil Red O dye (Sigma Aldrich, St. Louis, MO) for 30 min at room temperature. The cells were washed four times with distilled water. Images were collected using an Axiovert 40 CFL microscope (Carl Zeis AG, Oberkochen, Germany).

7. ELISA

The cytokines (MCP-1 and IL-6) were quantified by sandwich enzyme-linked immunosorbent assay (ELISA) Quantitation Kit (BD Biosciences Pharmingen, San Diego, CA, USA) according to the manufacturer's protocol. The total cytokine levels were quantified at 450 nm using a microplate reader (Molecular Devices), and calculated using a linear regression equation obtained from standard absorbance values.

8. RNA isolation and RT-PCR

The total RNA was isolated from the cells using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The primers used to amplify C/EBPα, PPARγ, aP2, MCP-1, IRS-1, GLUT4 and GAPDH are shown in Table 1. The PCR reaction was performed with a GeneAmp PCR System 9700 (Applied

Biosystems, Foster City, CA, USA). The PCR products were electrophoresed with 2% (w/v) agarose gels and stained with Ethidium Bromide (EtBr). GAPDH was used as a housekeeping gene for each experimental condition.

Table 1. Oligonucleotide primers used for RT-PCR in this study

Gene		Sequences (5′→3′)	Size(bp)	Accession
C/EBPα	sense	GTG TGC ACG TCT ATG CTA AAC CA	596	NM_001287523
	anti-sense	GCC GTT AGT GAA GAG TCT CAG TTT G		
PPARγ	sense	CGC TGA TGC ACT GCC TAT GA	122	NM_001127330
	anti-sense	TGC GAG TGG TCT TCC ATC AC		
aP2	sense	CCA ATG AGC AAG TGG CAA GA	133	NM_011547
	anti-sense	GAT GCC AGG CTC CAG GAT AG		
MCP-1	sense	GCC ATC ATA AAG GAG CCA TAA	104	U12470.1
	anti-sense	CTT TTG ATA GTT CAA TTC TTA GCA CA		
IRS-1	sense	TGG AAT TTA TAA ACC TCT TGA TGG A	103	NM_010570
	anti-sense	AAA CCT GTT CTC CAG TCT TTG C		
GLUT4	sense	CCT TCC TCT CTC TGA GCT CCT	301	NM_026877
	anti-sense	TTT TGT TCG TCG CTG TGG TA		
GAPDH	sense	CCA CAG TCC ATG CCA TCA C	568	NM_008084.3
	anti-sense	TCC ACC ACC CTG TTG CTG TA		

C/EBPα, CCAAT/enhancer binding protein, alpha; PPARγ, peroxisome proliferators-activated receptor gamma; aP2, adipocyte P2 ; MCP-1, macrophage chemoattractant protein-1; IRS-1, insulin receptor substrate-1; GLUT4, glucose transporter type 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

9. Western blotting

The cells were homogenized with an ice-cold lysis buffer consisting of 20 mmol/L Tris-HCl [pH 8.0], 150 mmol/L NaCl, 2 mmol/L ethylene diamine tetra acetic acid (EDTA), 1 mmol/L NaF, 1% Igepal CA-630, 1 mmol/L PMSF, 1 mmol/L Na_3VO_4, and protease inhibitor cocktail. After incubation on ice for 10 min, the homogenized suspension was centrifuged, and the supernatant was used to determine protein concentrations. Total proteins were separated with l0% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were then transferred to nitrocellulose transfer membranes (Whatman GmbH, Dassel, Germany). The membranes were blocked with 5% skim milk in a TBS-T buffer (10 mmol/L Tris-HCl [pH 7.5], 150 mmol/L NaCl, and 0.05% Tween 20) for 1 h and then incubated with antibodies (Cell Signaling Technologies, Beverly, MA, USA) to PPARγ, p-IRS-1(serine307), IRS-1, and β-actin. The primary antibodies (diluted 1/1000 in 5% skim milk in TBST) were incubated overnight at 4℃ and washed. The membranes were then incubated for 1 h with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG antibody (diluted 1/5000 in 5% skim milk in TBST) and immunoreactive bands were developed using an enhanced chemiluminescence regents (Pierce ECL Western Blotting Substrate; Pierce Biotechnology, Rockford, USA) according to the manufacturer's protocols.

10. Statistical analysis

Statistical analysis was performed with GraphPad Prism®5 package (GraphPad Software Inc., San Diego, Ca, USA). All data are expressed as mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc multiple comparison tests was used to assess statistical significance. $P < 0.05$ was considered significant.

Results

1. Effect of ACL on the cell viability of 3T3-L1 adipocytes

To avoid any cytotoxicity caused by ACL extract, we first investigated the effect of ACL extract on the cell viability in 3T3-L1 preadipocytes by MTT assay(Fig. 1). ACL extract at concentrations of 0.01, 0.1, 10, 100 and 500 μg/mL did not cause cell toxicity. Therefore, we used ACL extract at 100 μg/mL concentrations for subsequent studies of its anti-insulin resistance properties and action mechanism in the cells.

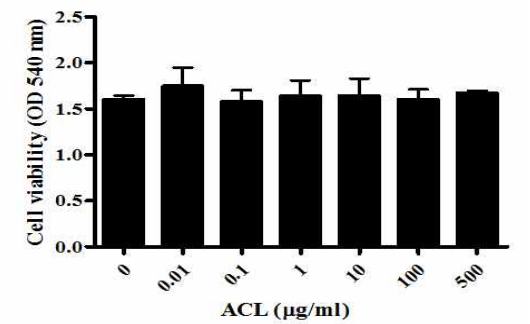

Fig. 1. Effects of ACL extract on the cell viability of 3T3-L1 preadipocytes. The cell viability was determined by MTT methods. 3T3-L1 preadipocytes was treated with the indicated concentrations of ACL for 24 h. Values are expressed as mean±SD. There is no significance.

2. ACL protects TNF-α-induced lipid droplets reduction

To investigate the effect of ACL extract on TNF-α -induced lipid droplets reduction, fully differentiated 3T3-L1 cells were pretreated with ACL extract at concentrations of 100 μg/mL for 24 h and then impaired by TNF-α. Adipogenesis and lipid accumulation were measured by Oil Red O staining for lipid droplet. As shown in Fig. 2, ACL extract significantly protected lipid droplets reduction impaired by TNF-α in 3T3-L1 cells. This data suggest that ACL extract protects TNF-α-induced lipid droplets reduction in mature adipocytes.

3. ACL suppresses TNF-α-mediated inflammatory cytokine production

To investigate the inhibitory effect of ACL extract on TNF-α-mediated inflammatory cytokine production, fully differentiated 3T3-L1 cells were pretreated with ACL extract at concentrations of 100 μg/mL for 24 h and then impaired by TNF-α. TNF-α increased the release of MCP-1 and IL-6 from 3T3-L1 cells. When treated with ACL extract at the concentration of 100 μg/mL for 24 h, the release of MCP-1 and IL-6 from 3T3-L1 cells significantly decreased compared to control(Fig. 3).

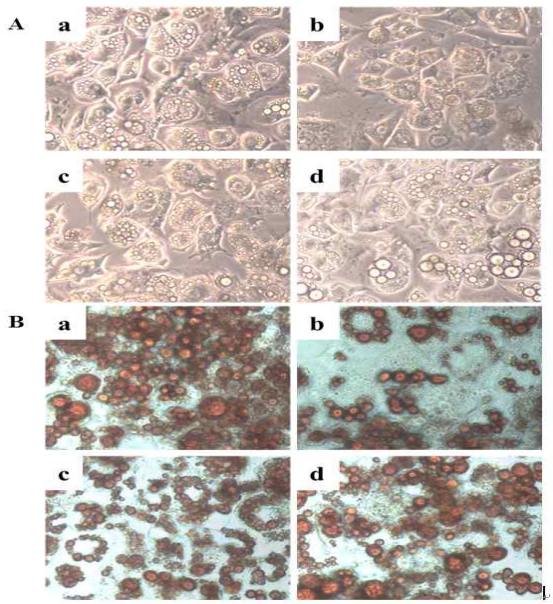

Fig. 2. Effects of ACL extract on the microscopic changes (A) and lipid droplet formation (B) in differentiated 3T3-L1 adipocytes. The fully differentiated 3T3-L1 adipocytes were treated with or without 100 μg/ml ACL for 24 h prior to 10 ng/ml TNF-α treatment for 24 h. (a) Untreated cells, (b) TNF-α treated cells, (c) TNF-α +ACL treated cells, (d) ACL treated cells. The cells treated with ACL increased the amounts and diameters of lipid droplet than TNF-α treated cells.

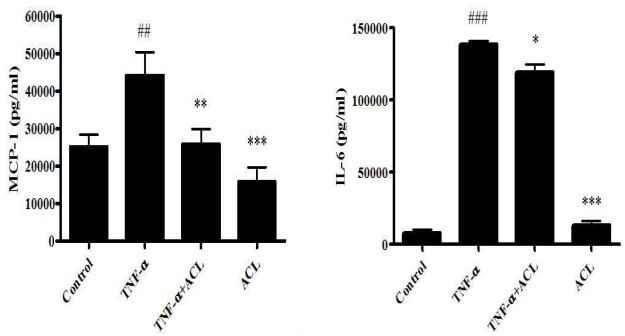

Fig. 3. Effects of ACL extract on the inflammatory cytokine levels in TNF-α-treated 3T3-L1 adipocytes. Cytokines were detected by ELISA. Values are expressed as mean±SD. ## p<0.01, ### p<0.001 vs control. *p<0.05, **p<0.01, ***p<0.001 vs TNF-α treated adipocytes. Dunnett's multiple comparison tests were used after one-way analysis of variance.

4. ACL regulates TNF-α-induced gene expression

We investigated the regulation of aP2, C/EBPα, PPARγ, MCP-1, GLUT4, and IRS-1 genes by ACL extract in 3T3-L1 adipocytes. Adipogenic differentiation-induced lipid accumulation is accompanied by induction of aP2, a mature adipocyte-specific marker and C/EBPα and PPARγ, the master transcription factor in adipocytes[13]. MCP-1, an adipocyte-proinflammatory cytokine, GLUT4, an insulin-regulated glucose transporter, and IRS-1, an insulin receptor substrate-1 are highly related to insulin resistance in adipose tissues[14,15]. Therefore, we investigated the expression of aP2, C/EBPα, PPARγ, MCP-1, GLUT4, and IRS-1 mRNA in 3T3-L1 adipocytes. As shown in Fig. 4, ACL extract protects lipolysis via upregulation of aP2, C/EBPα and PPARγ expression. ACL extract also improves inflammation-induced insulin resistance via downregulation of MCP-1 and upregulation of GLUT4 and IRS-1 expression.

This data suggest that ACL extract protects adipose dysfunction via regulation of lipolysis- and insulin resistance-related gene expression. Probably ACL extract influences the synthesis of these protein but also increases the gene expression.

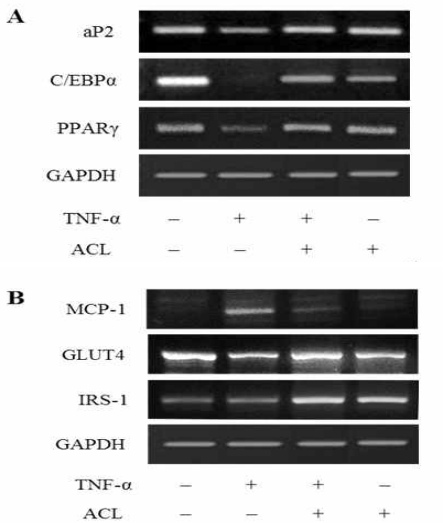

Fig. 4. Effects of ACL extract on insulin resistance-related genes in TNF-α-treated 3T3-L1 adipocytes. The fully differentiated 3T3-L1 adipocytes were treated with or without 100 μg/ml ACL for 24 h prior to 10 ng/ml TNF-α treatment for 24 h. The mRNA level was determinated by RT-PCR as described in 'Materials and Methods'.

5. ACL improves insulin signaling impaired by TNF-α

The study evaluated the effects of ACL extract on synthesis or phosphorylation of PPARγ and IRS-1 protein in the insulin signaling pathway in 3T3-L1 cells. Insulin signaling is initiated by the binding of insulin to the insulin receptor to activate IRS-1, which subsequently activates PI3K; the activation of PI3K results in the recruitment of GLUT4 to the cell surface[16]. In this study, ACL extract

improved TNF-α-induced PPARγ and IRS-1 suppression and suppressed TNF-α-induced IRS-1 serine 307 phosphorylation(Fig. 5). This represents at least part of the mechanism by which ACL extract enhances insulin sensitivity.

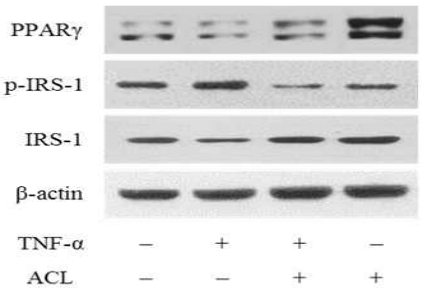

Fig. 5. Effects of ACL extract on insulin signaling molecules in TNF-α -treated 3T3-L1 adipocytes. The fully differentiated 3T3-L1 adipocytes were treated with or without 100 μg/ml ACL for 24 h prior to 10 ng/ml TNF-α treatment for 24 h. Total protein was analyzed by western blot using PPARγ, p-IRS-1, IRS-1, and β-actin as described in 'Materials and Methods'.

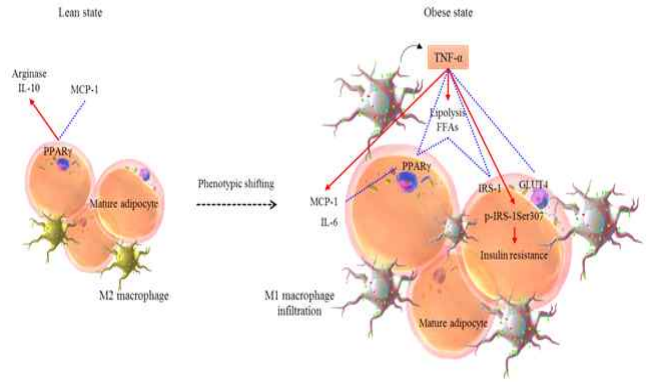

Fig. 6. Proposed model for adipocyte dysfunctions in obesity and insulin resistance. In the lean state, PPARγ maintains homeostasis and prevents classical activation of resident M2 (alternatively activated) macrophages, the secretion of chemokines such as MCP-1, and local inflammation to develop in mature adipocytes. M2 macrophages increase the production of arginase and the anti-inflammatory cytokine IL-10, participating in tissue repair and the attenuation of inflammatory responses. In the obese state, expansion of adipose tissue leads to adipocyte hypertrophy and the release of chemokines that induce increased recruitment of M1 (classically activated) macrophages from the blood stream. M1 macrophages are characterized by increased production of the pro-inflammatory cytokines TNF-α, which promote altered gene expression, inflammation and insulin resistance in adipocytes. These changes result in altered adipokine secretion, increased lipolysis and excess of circulating nonesterified fatty acids, which may eventually contribute to systemic insulin resistance. Red solid arrow indicates activation. Blue dotted line indicates inhibition.

Discussion

Obesity is characterized as a state of systemic low-grade inflammation induced by excessive nutrition, and is a major cause of insulin resistance[17]. The dysfunction of adipose tissue involved in glucose and lipid metabolism play a pivotal role in development of systemic insulin resistance[18]. TNF-α is an adipose tissue-derived proinflammatory cytokine that causes insulin resistance by enhancing adipocyte lipolysis and increasing the serine/threonine phosphorylation of IRS-1(Fig. 6)[19,20].

This study shows that ACL extract can prevent the TNF-α-induced inflammation and insulin resistance in 3T3-L1 adipocytes. As shown in Fig. 1, we found that ACL extract has not influence on the cell viability of 3T3-L1 preadipocytes at below 500 μg/ml concentration. Therefore, we used ACL extract at 100 μg/mL concentrations for subsequent studies of its anti-insulin resistance properties and action mechanism in the cells. As shown in Fig. 2, we found that TNF-α significantly induced the reduction of lipid droplets implying lipolysis in 3T3-L adipocytes, while ACL extract protected TNF-α-induced lipid droplets reduction. These results indicate that ACL extract may have actions to protect lipolysis in adipose tissue.

Adipocytes are one of the main source of proinflammatory cytokines, such as MCP-1 and IL-6. MCP-1 stimulates the recruitment of macrophages and dendritic cells, which further triggers the release of cytokines to exacerbate inflammation-induced insulin resistance[21]. IL-6 is recognized as an inflammatory mediator that causes insulin resistance by reducing the expression of GLUT4 and IRS-1[22]. Therefore, MCP-1 and IL-6 play a crucial role in the development of both inflammation and insulin resistance. As shown in Fig. 3, we showed that ACL extract inhibited the production of TNF-α-mediated proinflammatory cytokines, MCP-1 and IL-6 in 3T3-L1 adipocytes. These results indicate that ACL extract has potential to regulate chronic inflammation and insulin resistance in adipose tissue.

C/EBPα and PPARγ are transcriptional regulators of genes associated with adipogenesis, lipid metabolism, insulin sensitivity, energy expenditure, and insulin resistance. The expression of C/EBPα and PPARγ cross-regulate each other through a positive feedback loop and induce the expression of downstream target genes, such as aP2, which leads to the appearance of lipid droplets[13-15]. As shown in Fig. 4A, we found that ACL extract significantly promoted the expression of a mature adipocyte-specific marker aP2. We also showed that ACL extract enhanced the expression of the transcription factors C/EBPα and PPARγ. Therefore, these results demonstrate that ACL extract protects lipolysis by upregulation of a mature adipocyte-specific marker aP2 in 3T3-L1 adipocyte. Moreover, the transcription factors

C/EBPα and PPARγ are likely involved in this process. The increases of MCP-1 contribute to the pathogenesis of inflammation-induced insulin resistance during obesity[23,24]. Thus, we next investigated the gene expression of MCP-1, GLUT4, and IRS-1 in 3T3-1 adipocytes. As shown in Fig. 4B, we found that ACL extract significantly suppressed the expression of MCP-1. We also showed that ACL extract enhanced the expression of the GLUT4 and IRS-1. Therefore, these results demonstrate that ACL extract inflammation-induced insulin resistance by downregulation of an adipocyte-proinflammtory cytokine, MCP-1 via upregulation of GLUT4 and IRS-1 expression in 3T3-L1 adipocyte.

Next, we investigated whether ACL extract regulates TNF-α-reduced protein expression of PPARγ and IRS-1 and TNF-α-increased phosphorylation of IRS-1 Ser307. Phosphorylation of IRS-1 Ser307 precedes IRS-1 degradation, and phosphorylation of IRS-1 Ser307 has been recognized as an indicator molecule of insulin resistance[25-27]. As shown in Fig. 5, we found that ACL extract enhanced TNF-α-induced downregulation of PPARγ and IRS-1 protein expression. We also found that TNF-α increased phosphorylation of IRS-1 Ser307 in those cells associated with insulin resistance, while ACL extract improved both TNF-α-induced insulin resistance and over-phosphorylation of IRS-1 Ser307. Therefore, these results demonstrate that improved TNF-α-reduced expression of PPARγ and IRS-1 protein, indicating that ACL extract may improve insulin resistance by affecting a PPARγ - and IRS-1-mediated signal cascade.

In conclusion, we found that ACL extract could improve the TNF-α-induced inflammation and insulin resistance by elevating expression of PPARγ and IRS-1 via down-regulation of IRS-1 Ser307 phosphorylation. Thus, the water-based ACL extract can be an alternative to treat metabolic disease related to insulin resistance.

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References

1. Kahn, B.B., Flier, J.S. Obesity and insulin resistance. J. Clin. Invest. 106: 473-481, 2000.
2. Kim, J.A., Wei, Y., Sowers, J.R. Role of mitochondrial dysfunction in insulin resistance. Circ. Res. 102: 401-414, 2008.
3. Chen, X.H., Zhao, Y.P., Xue, M., Ji, C.B., Gao, C.L., Zhu, J.G., et al. TNF-alpha induces mitochondrial dysfunction in 3T3-L1 adipocytes. Mol. Cell. Endocrinol. 328: 63-69, 2010.
4. Anusree, S.S., Nisha, V.M., Priyanka, A., Raghu, K.G. Insulin resistance by TNF-alpha is associated with mitochondrial dysfunction in 3T3-L1 adipocytes and is ameliorated by punicic acid, a PPARgamma agonist. Mol. Cell. Endocrinol. 413: 120-128, 2015.
5. Cawthorn, W.P., Sethi, J.K. TNF-alpha and adipocyte biology. FEBS Lett 582: 117-131, 2008.
6. Daniele, G., Guardado Mendoza, R., Winnier, D., Fiorentino, T.V., Pengou, Z., Cornell, J., et al. The inflammatory status score including IL-6, TNF-alpha, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. Acta Diabetol. 51: 123-131, 2014.
7. Yeo, J., Kang, Y.M., Cho, S.I., Jung, M.H. Effects of a multi-herbal extract on type 2 diabetes. Chin Med. 6: 10, 2011.
8. Suneetha, W.J., Krishnakantha, T.P. Cardamom extract as inhibitor of human platelet aggregation. Phytother Res. 19: 437-440, 2005.
9. Roberts, L.D., Virtue, S., Vidal-Puig, A., Nicholls, A.W., Griffin, J.L. Metabolic phenotyping of a model of adipocyte differentiation. Physiol. Genomics. 39: 109-119, 2009.
10. Im, S.S., Kwon, S.K., Kang, S.Y., Kim, T.H., Kim, H.I., Hur, M.W., et al. Regulation of GLUT4 gene expression by SREBP-1c in adipocytes. Biochem. J. 399: 131-139, 2006.
11. Jitrapakdee, S., Slawik, M., Medina-Gomez, G., Campbell, M., Wallace, J.C., Sethi, J.K., et al. The peroxisome proliferator-activated receptor-gamma regulates murine pyruvate carboxylase gene expression in vivo and in vitro. J. Biol. Chem. 280: 27466-27476, 2005.
12. Wheatcroft, S.B., Kearney, M.T., Shah, A.M., Ezzat, V.A., Miell, J.R., Modo, M., et al. IGF-binding protein-2 protects against the development of obesity and insulin resistance. Diabetes 56: 285-294, 2007.
13. Ahn, J., Lee, H., Kim, S., Ha, T. Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/beta-catenin signaling. Am. J. Physiol., Cell Physiol. 298: C1510-1516, 2010.
14. Brannmark, C., Nyman, E., Fagerholm, S., Bergenholm,

L., Ekstrand, E.M., Cedersund, G., et al. Insulin signaling in type 2 diabetes: experimental and modeling analyses reveal mechanisms of insulin resistance in human adipocytes. J. Biol. Chem. 288: 9867-9880, 2013.

15. Chen, L., Chen, R., Wang, H., Liang, F. Mechanisms Linking Inflammation to Insulin Resistance. Int J Endocrinol. 2015: 508409, 2015.
16. Lee, O.H., Lee, H.H., Kim, J.H., Lee, B.Y. Effect of ginsenosides Rg3 and Re on glucose transport in mature 3T3-L1 adipocytes. Phytother Res. 25: 768-773, 2011.
17. Qatanani, M., Lazar, M.A. Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. 21: 1443-1455, 2007.
18. Anusree, S.S., Nisha, V.M., Priyanka, A., Raghu, K.G. Insulin resistance by TNF-alpha is associated with mitochondrial dysfunction in 3T3-L1 adipocytes and is ameliorated by punicic acid, a PPARgamma agonist. Mol. Cell. Endocrinol., 413: 120-128. 2015.
19. Hotamisligil, G.S. Inflammation and metabolic disorders. Nature 444: 860-867, 2006.
20. Maury, E., Brichard, S.M. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol. Cell. Endocrinol. 314: 1-16, 2010.
21. Kahn, S.E., Hull, R.L., Utzschneider, K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444: 840-846, 2006.
22. Serrano-Marco, L., Barroso, E., El Kochairi, I., Palomer, X., Michalik, L., Wahli, W., et al. The peroxisome proliferator-activated receptor (PPAR) beta/delta agonist GW501516 inhibits IL-6-induced signal transducer and activator of transcription 3 (STAT3) activation and insulin resistance in human liver cells. Diabetologia. 55: 743-751, 2012.
23. Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J. Clin. Invest. 116: 1494-1505, 2006.
24. Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. 112: 1821-1830, 2003.
25. Aguirre, V., Werner, E.D., Giraud, J., Lee, Y.H., Shoelson, S.E., White, M.F. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J. Biol. Chem. 277: 1531-1537, 2002.
26. Gao, Z., Hwang, D., Bataille, F., Lefevre, M., York, D., Quon, M.J., et al. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. The J. Biol. Chem. 277: 48115-48121, 2002.
27. Zhande, R., Mitchell, J.J., Wu, J., Sun, X.J. Molecular mechanism of insulin-induced degradation of insulin receptor substrate 1. Mol. Cell. Endocrinol. 22: 1016-1026, 2002.