ISSN (Online) 2287-3406
Journal of Life Science **2023** Vol. 33. No. 11. 859~867 DOI : https://doi.org/10.5352/JLS.2023.33.11.859

Lupeol Improves TNF-α Induced Insulin Resistance by Downregulating the Serine Phosphorylation of Insulin Receptor Substrate 1 in 3T3-L1 Adipocytes

Hyun Ah Lee and Ji Sook Han*

Department of Food Science and Nutrition, Pusan National University, Busan 46241, Republic of Korea

Received September 1, 2023 /Revised September 26, 2023 /Accepted October 17, 2023

Lupeol is a type of pentacyclic triterpene that has been reported to have therapeutic effects for treating many diseases; however, its effect on insulin resistance is unclear clear. This study examined the inhibitory effect of lupeol on the serine phosphorylation of insulin receptor substrate-1 in insulin resistance-induced 3T3-L1 adipocytes. 3T3-L1 cells were cultured and treated with tumor necrosis factor-α (TNF-α) for 24 hours to induce insulin resistance. Cells treated with different concentrations of lupeol (15 μM or 30 μM) or 100 nM of rosiglitazone were incubated. Then, lysed cells underwent western blotting. Lupeol exhibited a positive effect on the negative regulator of insulin signaling and inflammation-activated protein kinase caused by TNF-α in adipocytes. Lupeol inhibited the activation of protein tyrosine phosphatase-1B (PTP-1B)－a negative regulator of insulin signaling－and c-Jun N-terminal kinase (JNK); it was also an inhibitor of nuclear factor kappa-B kinase (IKK) and inflammation-activated protein kinases. In addition, Lupeol downregulated serine phosphorylation and upregulated tyrosine phosphorylation in insulin receptor substrate-1. Then, the downregulated phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway was activated, the translocation of glucose transporter type 4 was stimulated to the cell membrane, and intracellular glucose uptake increased in the insulin resistance-induced 3T3-L1 adipocytes. Lupeol may improve TNF-α-induced insulin resistance by downregulating the serine phosphorylation of insulin receptor substrate 1 by inhibiting negative regulators of insulin signaling and inflammation-activated protein kinases in 3T3-L1 adipocytes.

Key words : GLUT4, insulin resistance, Lupeol, PI3K/AKT pathway, TNF-α

Introduction

Obesity is accompanied by fat accumulation and inflammatory reactions in adipose tissue, leading to insulin resistance [6]. Insulin is known as a hormone that maintains glucose homeostasis, and insulin resistance impairs insulin signaling pathway and inhibits intracellular glucose uptake [26, 29]. Reduction of intracellular glucose uptake leads to increased blood glucose, and hyperglycemia may cause diabetes and diabetic complications [8]. Insulin resistance by obesity results from increased secretion of proinflammatory cytokines in activated macrophages and adipocytes by the deposition of macrophages in adipose tissue [10]. Tumor necrosis factor-α (TNF-α), a proinflammatory cytokine, activates protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signal transduction, and c-Jun N-terminal kinase (JNK) and IκB kinase (IKK), inflammation-activated protein kinases [23, 32]. It impairs the insulin signaling pathway by increasing serine phosphorylation and decreasing tyrosine phosphorylation in insulin receptor substrate-1 (IRS-1) [12, 27].

In the insulin signaling pathway, impaired IRS-1 inhibits the activation of the subkinase phosphoinositide 3-kinase (PI3K). Inhibition of PI3K activation also suppresses the activation of two downstream targets, serine/threonine protein kinase B (AKT), and atypical protein kinase C isoforms ζ and λ (PKC ζ / λ). Then, the translocation of glucose transporter type 4 (GLUT4) to the cell membrane decreases and intracellular glucose uptake is down-regulated [1, 5, 19]. Therefore, several studies have been conducted to improve insulin resistance by TNF-α secretion.

Lupeol ($C_{30}H_{50}O$) is a pentacyclic lupane-type triterpene, and is included in medicinal plants such as crataeva nurvala and birch barks. A 30-carbon skeleton including of four-six membered rings is the basis for pentacyclic lupane-type triterpenes. Lupeol has been reported to have therapeutic ef-

***Corresponding author**
Tel : +82-51-510-2836, Fax : +82-51-583-3648
E-mail : hanjs@pusan.ac.kr

fects for diseases such as inflammation, cancer, heart disease, arthritis, and hepatic toxicity [2, 7]. However, the effect of lupeol on insulin resistance is not yet clear. Therefore, this study investigated the improvement effect of lupeol on TNF-α -induced insulin resistance by down-regulating serine phosphorylation of insulin receptor substrate 1 through inhibiting negative regulators of insulin signaling and inflammation-activated protein kinases in 3T3-L1 adipocytes.

Materials and Methods

Materials

Lupeol (99%) was purchased from Sigma Aldrich (St. Louis, MO, USA) and dissolved in phosphate buffered saline (PBS). Tumor necrosis factor-alpha (TNF-α), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and bovine calf serum (BCS) were purchased from Sigma (St. Louis, MO, USA). 2-Deoxy-2-[(7-nitro-2, 1, 3-benzoxadiazol-4-yl) amino]-D-glucose (2-NBDG) was purchased from Invitrogen (Carlsbad, California, USA). Mouse 3T3-L1 pre- adipocyte cells were purchased from Korean Cell Line Bank (Seoul, Korea). All chemicals were of analytical grade and were used without any further purification.

Cell culture and differentiation

3T3-L1 preadipocytes (KCLB, Seoul, Korea) were cultured in high glucose (4.5 mM) Dulbecco's modified Eagle medium containing 10% fetal bovine serum at 37°C temperature and 5% CO_2. Cultured 3T3-L1 preadipocytes were differentiated into adipocytes in the following way. 2 days after confluence, the culture medium was replaced with new Dulbecco's modified Eagle medium with 10% fetal calf serum, 0.5 mM isobutylmethyl xanthine, 1 μM dexamethasone, and 10 μg/ml insulin. Afterwards, fresh 10% FBS DMEM with 10 μg/ml insulin was treated to the cells for an additional 24 hr, and maintained for 8 days [16].

Glucose uptake assay

Glucose uptake was conducted using the 2-[N-(7-nitobenz-2-oxa-1,3-diazol-4-yl) Amino]-2 deoxyglucose (2-NBDG) screening system, as described previously with partial modifications. Differentiated 3T3-L1 adipocytes of 1×10^4 density were induced insulin resistance using TNF-α 50 ng/ml into each well of 96-well plates for 24 hr. After that, the cells were treated rosiglitazone 100 nM or several lupeol concentrations (0, 10, 20, 25, 30, 50 μM) into each well of 96-well plates for 24 hr. The cells were stimulated with insulin (100 nM) for 20 min at 37°C in Krebs-Ringer phosphate buffer solution (4.7 mM KCl, 128 mM NaCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, and 10 mM $NaPO_4$, pH 7.4). Glucose uptake was initiated by adding 2-NBDG (10 μM) to each well. 1 hr later the supernatant was removed, uptake of 2-NBDG was measured using a Multilabel Counter (Perkin Elmer, MA, USA) set at an excitation and emission wavelengths of 485 and 535 nm.

Cell viability

The MTT assay was used to estimate cell viability. 3T3-L1 cells were seeded at the concentration of 1×10^4 cells/well in 96-well plates and pre-incubated in humidified atmosphere (37°C, 5% CO_2) for 24 hr. Then, 3T3-L1-adipocytes were treated with TNF-α for 24 hr to induce insulin resistance. Next, different concentrations of lupeol (the same concentration lupeol range as the glucose uptake assay) or rosiglitazone 100 nM-treated cells were incubated in same humidified atmosphere for 24 hr. To each well, filtered 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) solution and was added and incubated in same humidified atmosphere for 4 hr. The absorbance was measured at 540 nm by using a microplate reader.

Western-blot analysis

3T3-L1 cells were washed twice with ice-cold phosphate-buffered saline (PBS) and collected in RIPA buffer [Tris-HCl (50 mM), NaCl (150 mM), EDTA (1 mM), Triton X-100 (1%), sodium deoxycholate (1%), sodium dodecyl sulfate (0.1%), phenylmethylsulfonyl fluoride (1 mM), aprotinin (10 μg/ml), leupeptin (10 μg/ml), sodium orthovanadate (0.1 mM); pH 7.4] for whole protein extraction from 3T3-L1 adipocytes. After sonication and centrifugation at 13,000 rpm for 15 min at 4°C, a bicinchoninic acid (BCA) Protein Assay Kit was used to determine the protein content in the resulting supernatant. The lysate containing 20 μg protein was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Isolated proteins were transferred electrophoretically to a pure nitrocellulose membrane, blocked with a 5% skimmed milk solution for 1 hr, and then incubated with primary antibodies [PTP1B, SOCS3, p-JNK, JNK, IκBα (Santa Cruz Biotechnology, CA, USA), phospho-IRS-1Ser 307, IRS-1, PI3K, phospho-Akt Ser 473, Akt, AMPK, GLUT4 (Abcam, Cambridge, UK), phospho-AMPK Thr172, ACC (Cell Signaling Technology, Beverly, MA, USA), phospho-IRS-1Tyr 612 (Thermo Fisher Scientific, Rockford, IL, USA), phospho-ACCSer79 (EMD Millipore, Billerica, MA, USA)]

overnight at 4°C. After rinsing, the blots were cultured with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG (secondary antibody) for 1 hr at room temperature. The antigen-antibody complexes were visualized using enhanced chemiluminescence western blotting detection reagents and detected by a Luminoimage Analyzer LAS-1000 plus (Fujifilm, Tokyo, Japan). The band density was determined using an image analyzer (Multi Gauge V3.1, Fujifilm, Valhalla, NY, USA) and normalized to the β-actin chemiluminescence signal for relative total and nuclear protein quantification.

Statistical analysis

Data were determined as the mean ± standard error of three experiments. The statistical analysis was conducted using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The differences were evaluated by the one-way ANOVA test, followed by the post hoc Duncan's multiple range tests.

Results

Lupeol increases glucose uptake in insulin-resistant adipocytes

This study examined a 2-deoxyglucose uptake assay to find out the effect of lupeol on glucose uptake in 3T3-L1 adipocytes with insulin resistance. Glucose uptake was decreased 0.32-fold compared to untreated control cells in TNF-α treated cells, but increased concentration-dependently with lupeol treatment (Fig. 1A). At 10, 20, 25, 30, and 50 μM concentration of lupeol, glucose uptake was significantly increased 0.47-, 0.69-, 0.78-, 0.84-, and 0.88-fold in comparison with 0.32-fold in cells treated with TNF-α, respectively. The data showed that lupeol could be effective in enhancing uptake of glucose in 3T3-L1 adipocytes with insulin resistance.

Lupeol increases cell viability in insulin-resistant adipocytes

3T3-L1 cells induced insulin resistance by TNF-α were treated with various concentrations of lupeol. And then cell viability was measured by MTT assay (Fig. 1B). The data showed that lupeol could enhance cell viability by alleviating the cytotoxicity induced by TNF-α in 3T3-L1 adipocytes.

Lupeol decreases the activation of PTP1B and JNK in insulin-resistant adipocytes

To investigate the mechanism that lupeol improves TNF-α-induced insulin resistance in 3T3-L1 adipocytes, we investigated the activation of negative regulator of insulin signaling

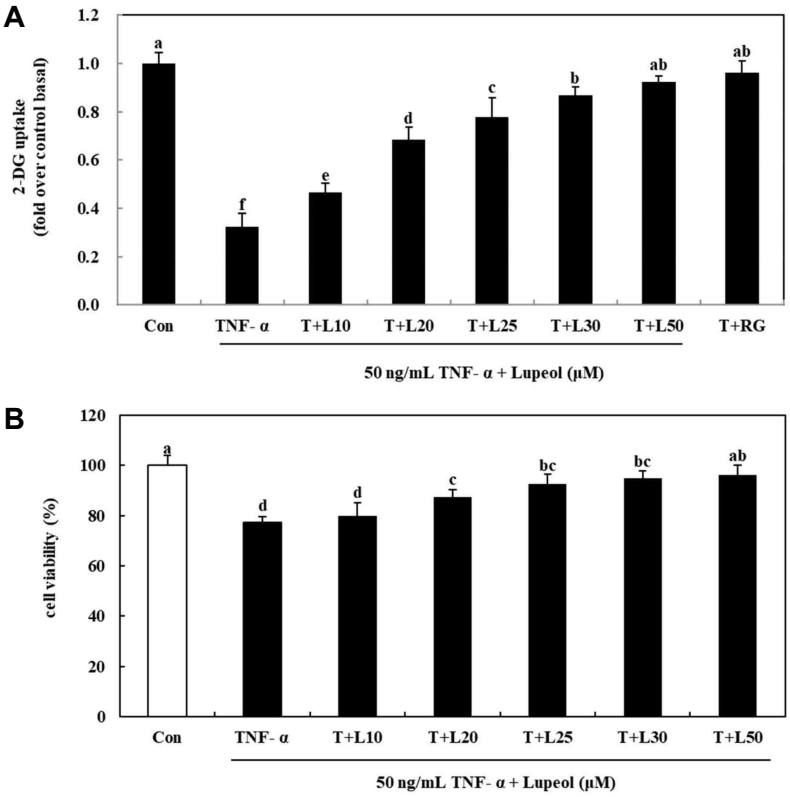

Fig. 1. (A) 2-deoxy glucose uptake and (B) cell viability of lupeol in insulin-resistant adipocytes. TNF-α of 50 ng/ml was treated in 3T3-L1 adipocytes for 24 hr. After that, lupeol of 10 - 50 μM or rosiglitazone (RG) of 100 nM were treated in the cells for 24 hr. (A) 2-deoxy glucose (DG) uptake, (B) cell viability. Each value is shown as the mean ± standard deviation (n=3). [a-f]Values composed of different alphabets indicate significant differences (p<0.05) by Duncan's multiple range test.

and inflammation-activated protein kinases, which were insulin resistance inducing factors (Fig. 2). Lupeol significantly inhibited the activation of PTP1B known as negative regulator of insulin signaling in insulin-resistant adipocytes. The activation of PTP1B was inhibited by 15 and 30 μM lupeol to 3.6- and 2.5-fold compared to TNF-α value (4.1- fold of control), respectively. Also, Lupeol significantly inhibited the activation of JNK known as inflammation-activated protein kinase in insulin-resistant adipocytes. At the same concentration of lupeol, the phosphorylation of JNK was significantly decreased to 1.9- and 1.6-fold compared to TNF-α value (2.6-fold of control), respectively. Lupeol also significantly prevented the degradation of IκBα by inhibiting the activation of IKK in insulin-resistant adipocytes, and then the IκBα expression was significantly increased to 0.7- and 0.8-fold compared to TNF-α value (0.3-fold of control), respectively. These data suggested that lupeol could inhibit the activation of PTP1B, JNK and IKK in insulin-resistant adipocytes.

Lupeol decreases serine phosphorylation and increases tyrosine phosphorylation of IRS-1 in insulin-resistant adipocytes

To investigate the effect of lupeol on the improvement of insulin signaling pathway impaired by TNF-α, we investigated the expression of serine and tyrosine phosphorylation in IRS-1 (Fig. 3). Lupeol down-regulated serine phosphorylation and up-regulated tyrosine phosphorylation in IRS-1. Treatment of 15 and 30 μM lupeol reduced serine phosphorylation of IRS-1 to 2.3- and 1.7-fold compared to TNF-α value (2.8- fold of control), respectively. At the same concentration of lupeol, tyrosine phosphorylation of IRS-1 was enhanced to 0.3- and 0.8-fold compared to value (0.2-fold of control), respectively. These data indicated that lupeol could improve impaired insulin signaling in insulin-resistant adipocytes.

Lupeol increases the activation of PI3K and AKT in insulin-resistant adipocytes

To investigate the improvement effect of lupeol on the im-

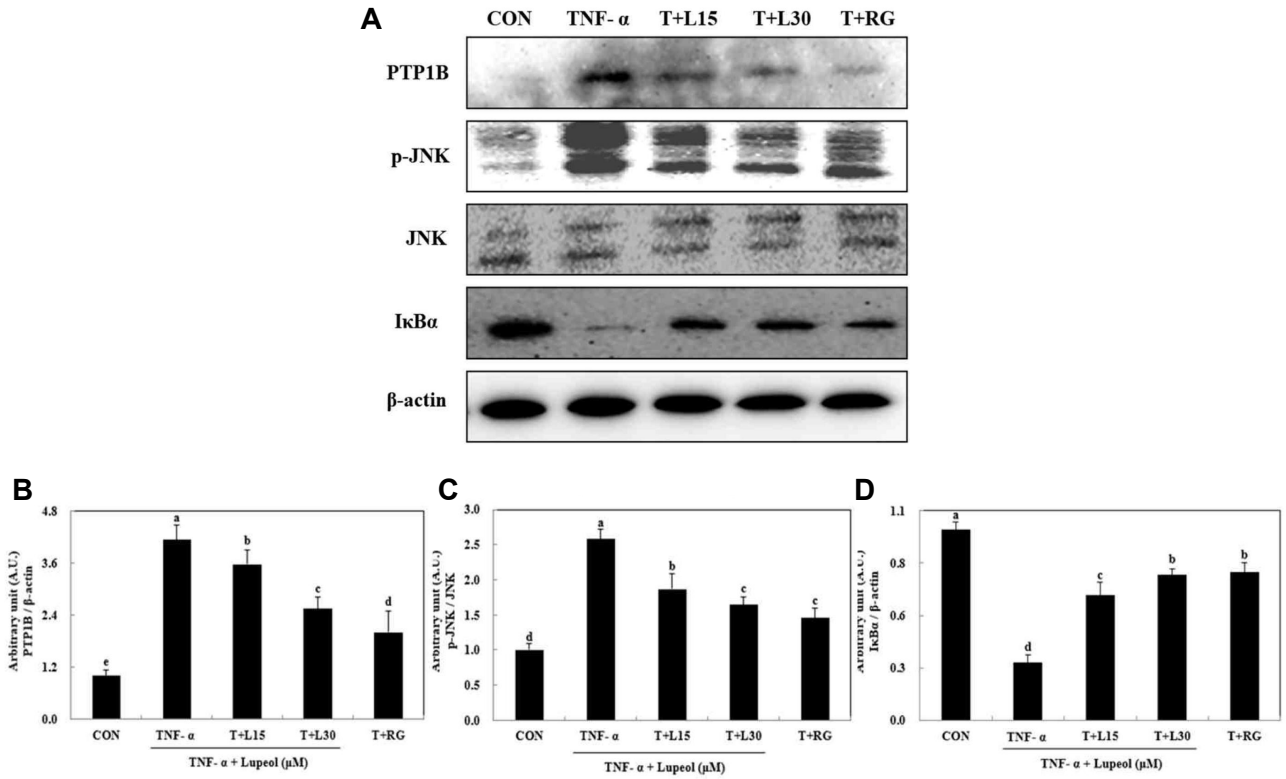

Fig. 2. Lupeol decreases the activation of PTP1B and JNK and increases the expression of IκBα in insulin-resistant adipocytes. Lupeol of 15, 30 μM or rosiglitazone of 100 nM were treated in 3T3-L1 adipocytes with insulin resistance for 24 hr, after that, (A) lysed cells were performed to western blotting. (B) Expression value of PTP1B, (C) Phosphorylation value of JNK, (D) Expression value of IκBα. Each value is shown as the mean ± standard deviation (n=3). [a-e]Values composed of different alphabets indicate significant differences (p<0.05) by Duncan's multiple range test.

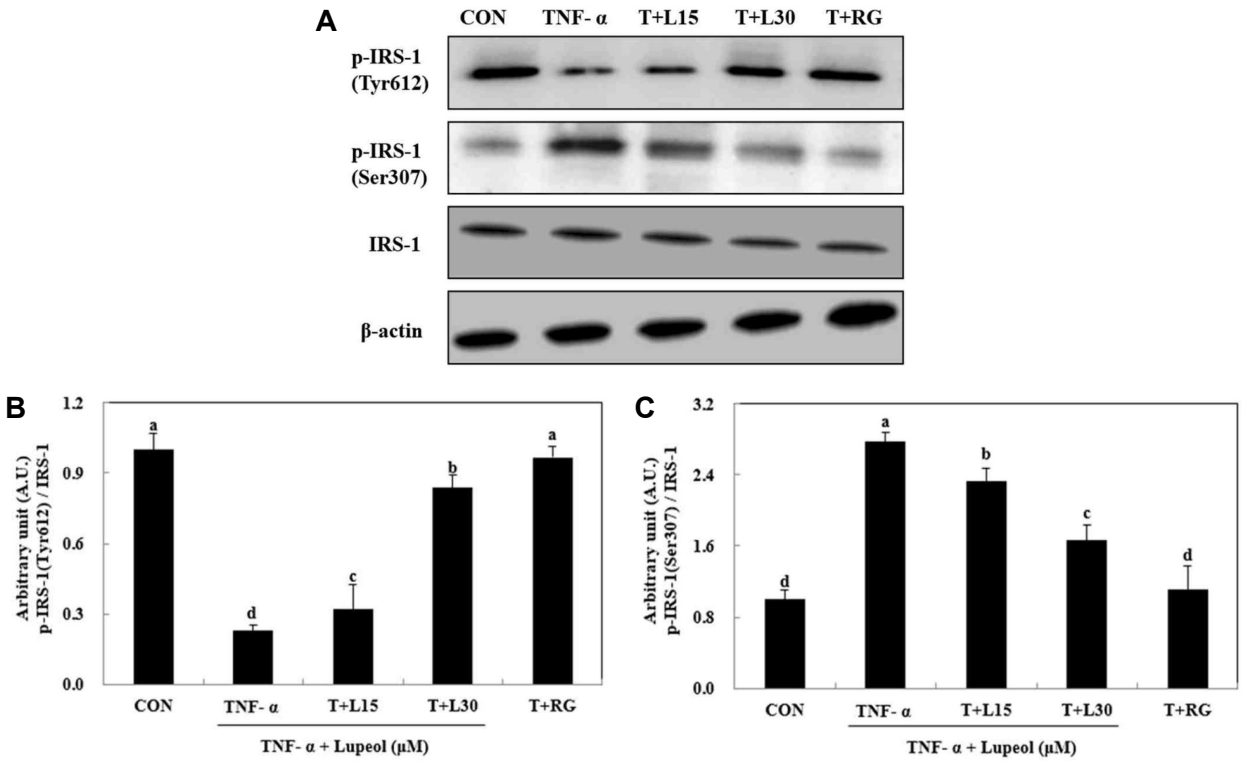

Fig. 3. Lupeol increases tyrosine phosphorylation and decreases serine phosphorylation of IRS-1 in insulin-resistant adipocytes. Lupeol of 15, 30 μM or rosiglitazone of 100 nM were treated in 3T3-L1 adipocytes with insulin resistance for 24 hr, after that, (A) lysed cells were performed to western blotting. (B) Phosphorylation value of IRS-1 (Tyr612), (C) Phosphorylation value of IRS-1 (Ser307). Each value is shown as the mean ± standard deviation (n=3). $^{a-d}$Values composed of different alphabets indicate significant differences (p<0.05) by Duncan's multiple range test.

paired insulin signaling pathway, we investigated the activation of PI3K, AKT and PKC ζ/λ (Fig. 4). The activation of PI3K and AKT, the sub-kinases of IRS-1, were also increased by lupeol, but there was no significant difference in PKC ζ/λ. The activation of PI3K by 15 and 30 μM lupeol increased to 0.6- and 0.9-fold compared to TNF-α value (0.2-fold of control). The phosphorylation of AKT also enhanced by lupeol to 0.4- and 0.8-fold compared to TNF-α value (0.3-fold of control). These data indicated that lupeol could improve the impaired PI3K / AKT pathway in insulin-resistant adipocytes.

Lupeol increases the expression of plasma membrane GLUT4 in insulin-resistant adipocytes

To confirm the improvement effect of lupeol on the impaired insulin signaling pathway, we examined the translocation of GLUT4 to the cell membrane (Fig. 5). Treatment of lupeol significantly stimulated translocation of GLUT4 to plasma membrane (PM) in insulin-resistant adipocytes. The expression of PM-GLUT4 up-regulated by 15 and 30 μM lupeol to 0.7- and 0.9-fold compared to TNF-α value (0.5-fold of the control). It showed that lupeol could stimulate the translocation of GLUT4 to the plasma membrane via PI3K/AKT pathway.

Discussion

Studies have reported that natural compounds are effective in preventing and treating disease as much as drugs, but without side effects [4]. Triterpenoid compounds are known to have various beneficial effects including anti-diabetic and anti-inflammatory activities [22]. Lupeol is a pentacyclic lupane-type triterpene, and has pharmacological activities and therapeutic effects [17]. However, the effect of lupeol on insulin resistance in adipocytes is not yet known. Therefore, this study was designed to investigate the improvement effect of lupeol on TNF-α-induced insulin resistance by down-regulating serine phosphorylation of insulin receptor substrate 1 through suppressing negative regulators of insulin signaling and inflammation-activated protein kinases in 3T3-L1 adipocytes. Obesity and diabetes are known to reduce intracellular glucose uptake by causing insulin resistance in adipocytes

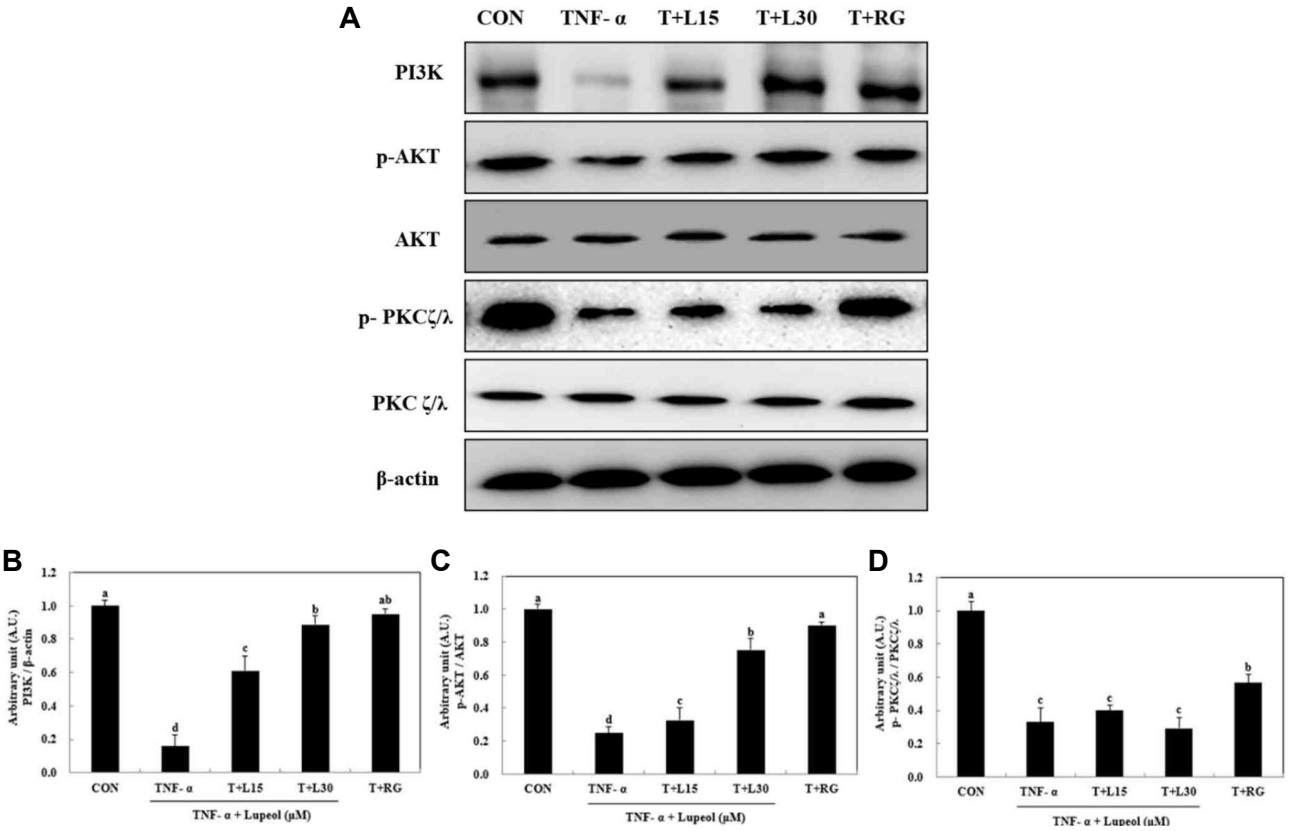

Fig. 4. Lupeol increases the expression of PI3K and AKT in insulin-resistant adipocytes. Lupeol of 15, 30 μM or rosiglitazone of 100 nM were treated in 3T3-L1 adipocytes with insulin resistance for 24 hr, after that, (A) lysed cells were performed to western blotting. (B) Expression value of PI3K, (C) Phosphorylation value of AKT, (D) Phosphorylation value of PKC ζ/λ. Each value is shown as the mean ± standard deviation (n=3). [a-d]Values composed of different alphabets indicate significant differences (p<0.05) by Duncan's multiple range test.

[24]. Insulin resistance results from an increase in proinflammatory cytokines such as TNF-α in activated macrophages and adipocytes by the deposition of macrophages in adipose tissue [3]. TNF-α binds to transmembrane-spanning receptors TNFR1 and TNFR2 with high affinity, exerting most of the action of molecular mechanism [28]. It increases the activation of PTP1B, a negative regulator of insulin signaling, and JNK and IKK, inflammation-activated protein kinases [8,10]. Activation of these impairs IRS-1 by increasing serine phosphorylation and decreasing tyrosine phosphorylation [23, 32]. In the impaired insulin signaling pathway, PI3K, AKT, and PKC ζ/λ are suppressed, and finally GLUT4 translocation to the plasma membrane and intracellular glucose uptake decreases [5, 12, 27].

Lupeol significantly increased glucose uptake and cell viability in insulin-resistant adipocytes. It showed that lupeol could alleviate cytotoxicity by TNF-α and increase glucose uptake in insulin-resistant adipocytes, which played an important role in reducing hyperglycemia. Glucose uptake might be enhanced by improving impaired IRS-1 through inhibition of negative regulators of insulin signaling and inflammation-activated protein kinases in insulin-resistant adipocytes [23, 32]. In the insulin signaling pathway, increased tyrosine phosphorylation and reduced serine phosphorylation in IRS-1 activate PI3K and phosphorylate AKT. It stimulates the translocation of GLUT4 to the plasma membrane and increases intracellular glucose uptake [1, 5].

To understand the mechanism of lupeol action enhancing glucose uptake in insulin-resistant adipocytes, we examined negative regulator of insulin signaling and inflammation-activated protein kinases. Lupeol significantly inhibited the activation of PTP1B known as negative regulator of insulin signal transduction in insulin-resistant adipocytes. Also, Lupeol significantly inhibited the activation of JNK and IKK known as inflammation-activated protein kinases in insulin-resistant adipocytes. Inhibition of IKK activation was confirmed by a decrease in the degradation of IκBα by lupeol. These data suggested that lupeol could alleviate TNF-α-induced insulin

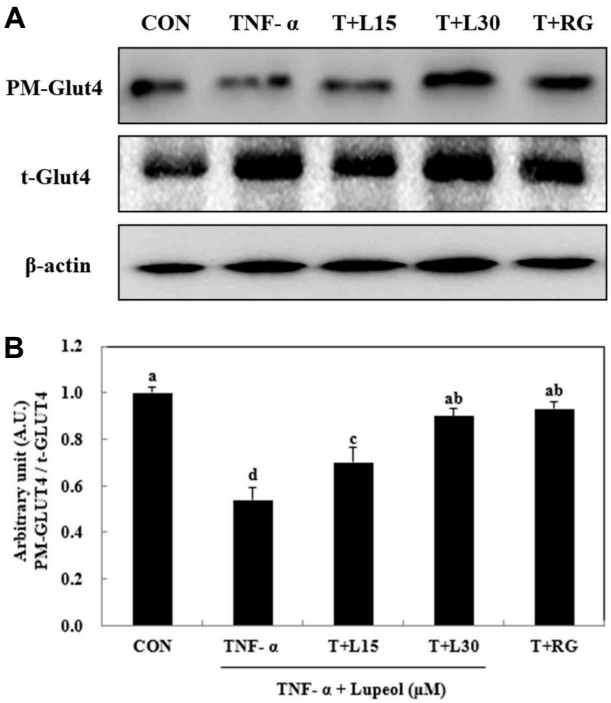

Fig. 5. Lupeol increases the expression of plasma membrane GLUT4 in insulin-resistant adipocytes. Lupeol of 15, 30 μM or rosiglitazone of 100 nM were treated in 3T3-L1 adipocytes with insulin resistance for 24 hr, after that, (A) lysed cells were performed to western blotting. (B) Expression value of PM-GLUT4. Each value is shown as the mean ± standard deviation (n=3). $^{a-d}$Values composed of different alphabets indicate significant differences (p<0.05) by Duncan's multiple range test.

resistance by inhibiting the activation of PTP1B, JNK, and IKK in 3T3-L1 adipocytes.

Lupeol belongs to the pentacyclic lupane-type triterpene and is a bioactive compound present in medicinal plants such as *crataeva nurvala* and birch barks [19]. It was reported that lupane-type triterpene inhibited the activation of PTP1B by interacting with its hydrophobic allosteric site [14]. The hydrophobic interaction between the lupane-type triterpene and PTP1B was strengthened by α7 as regulatory helix in the allosteric site [20, 21]. α7 participated in the formation of PTP1B-ligand binding with lupane-type triterpene and contributed to their allosteric binding mode. In addition, α7 in PTPIB provided non-polar residue. Lupane-type triterpene also contained a non-polar pentacyclic structure, so it inhibited the activation of PTPIB by forming protein-ligand complex through hydrophobic interaction with the hydrophobic tunnel formed by α7 [13]. Triterpenoids affected a number of signaling pathways, of which they exhibited anti-inflammatory activity by blocking TNF-induced NF-κB activation through inhibition of IKK [18, 25]. It has been reported that the anti-inflammatory' activity was due to the presence of the hydroxyl group in the pentacyclic triterpene [11, 30]. Thus, we assume that non-polar pentacyclic structure or hydroxyl group of lupeol may partly contribute to inhibit the activation of PTP1B or IKK in insulin resistant adipocytes, respectively.

To clarify the effect of lupeol on the improvement of impaired insulin signal pathway, we investigated insulin signal pathway in insulin resistant adipocytes. Lupeol significantly improved insulin resistance by up-regulating tyrosine phoshorylation and down-regulating serine phosphorylation in IRS-1. Impaired insulin signal pathway was also improved by increasing the activation of PI3K and AKT, the sub-kinases of IRS-1 by lupeol. Thus, the expression of PM-GLUT4 was significantly enhanced and intracellular glucose uptake was promoted by lupeol in insulin-resistant adipocytes. In recent studies, triterpenes activated the IRS-1/PI3K/Akt signal pathway and improved glucose homeostasis [33]. Corosolic acid, a natural triterpenoid, effectively restored IRS-1 tyrosine phosphorylation by inhibiting IRS-1 serine phosphorylation, and improved insulin resistance by activating insulin signaling pathway through Akt phosphorylation in adipose tissue [31]. Two triterpenoids from *Cyclocarya paliurus Iljinsk* activated insulin-mediated Akt phosphorylation by regulating IRS-1 serine/tyrosine phosphorylation, thereby stimulating glucose uptake through improved insulin resistance [34]. The ability of triterpenoids to improve insulin resistance was related to the location and number of hydroxyl groups in their structure [16]. The improvement effect on insulin resistance was greater, especially when there were more hydroxyl groups or hydroxyl group at the C3 position [15]. Lupeol also contains a hydroxyl group at the C3 position, showing its improved effect on insulin resistance.

In this study, lupeol improved TNF-α-induced insulin resistance by down-regulating serine phosphorylation and up-regulating tyrosine phosphorylation of IRS-1 through inhibition of negative regulator of insulin signaling and inflammation-activated protein kinases in 3T3-L1 adipocytes. These results suggested that lupeol could be useful as a potential therapeutic agent to improve insulin resistance.

Acknowledgment

This work was supported by a 2-year research grant of Pusan National University.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Bonizzi, G., Piette, J., Schoonbroodt, S., Merville, M. P. and Bours, V. 1999. Role of the protein kinase C lambda/iota isoform in nuclear factor-kappaB activation by interleukin-1beta or tumor necrosis factor-alpha: cell type specificities. *Biochem. Pharmacol.* **57**, 713-720.
2. Cháirez-Ramírez, M. H., Moreno-Jiménez, M. R., González-Laredo, R. F., Gallegos-Infante J. A. and Rocha-Guzmán, N.E. 2016. Lupane-type triterpenes and their anti-cancer activities against most common malignant tumors: A review. *EXCLI. J.* **15**, 58-771.
3. Coats, B. R., Schoenfelt, K. Q., Barbosa-Lorenzi, V. C., Eduard P., Chang C., Alexandria H., Guolin Z., Sully F., Lijie Z., Ben, A. H., Abigail, S. H., Ajay, M. S., Catherine A. R., Matthew, J. B., Christopher, J. R., Frederick, R. M. and Lev, B. 2017. Metabolically activated adipose tissue macrophages perform detrimental and beneficial functions during diet-induced obesity. *Cell Rep.* **20**, 3149-3161.
4. Cragg, G. M.and Newman, D. J. 2013. Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta* **1830**, 3670-3695.
5. Cristina de A., Teresa T., Rosario H. and Margarita L.. 2004. Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitory kappa B kinase in a p38 MAPK-dependent manner. *J. Biol. Chem.* **279**, 17070-17078.
6. Fuster, J. J., Ouchi, N., Gokce, N. and Kenneth W. 2016. Obesity-induced changes in adipose tissue microenvironment and their impact on cardiovascular disease. *Circ. Res.* **118**, 1786-1807.
7. Gallo, M. B. C. and Sarachine, M. J. 2009. Biological Activities of Lupeol. *Int. J. Biomed. Pharm. Sci* **3**, 46-66.
8. Giugliano, D., Ceriello, A. and Paolisso, G. 1996. Oxidative stress and diabetic vascular complications. *Diabetes Care* **19**, 257-267.
9. Green, H. and Kehinde, O. 1975. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. *Cell* **5**, 19-27.
10. Hill, A. A., Reid, B. W. and Hasty, A. H. 2014. A decade of progress in adipose tissue macrophage biology. *Immunol Rev.* **262**, 134-152.
11. Huang R. Z., Jin, L., Wang, C. G., Xu, X. J., Du, Y., Liao, N., Min ji, Liao, Z. X. and Wang. H. S. 2018. A pentacyclic triterpene derivative possessing polyhydroxyl ring A suppresses growth of HeLa cells by reactive oxygen species-dependent NF-κB pathway. *Eur. J. Pharmacol.* **838**, 157-169.
12. Jiang, S. and Messina, J. L. 2011. Role of inhibitory κB kinase and c-Jun NH2-terminal kinase in the development of hepatic insulin resistance in critical illness diabetes. *Am J Physiol Gastrointest Liver Physiol.* **301**, 454-463.
13. Jin, T., Yu, H. and Huang, X. F. 2016. Selective binding modes and allosteric inhibitory effects of lupane triterpenes on protein tyrosine phosphatase 1B. *Sci Rep* **6**, 20766.
14. Jose, M. C., Angeles, G., Teresa, D. Mirela, R. and Jose, A. C. 2013. Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. *Diabetes* **62**, 1791-1799.
15. Jung, S. H., Ha, Y. J., Shim, E. K., Choi, S. Y., Jin J. L., Yun-Choi, H. S. and Lee, J. R. 2007. Insulin-mimetic and insulin-sensitizing activities of a pentacyclic triterpenoid insulin receptor activator. *Biochem. J.* **403**, 243-250.
16. Khan, M. F., Maurya, C. K., Dev, K., Arha, D., Rai, A. K., Tamrakar, A. K. and Maurya, R. 2014. Design and synthesis of lupeol analogues and their glucose uptake stimulatory effect in L6 skeletal muscle cells. *Bioorg. Med. Chem. Lett.* **24**, 2674-2679.
17. Laszczyk, M. N. 2009. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. *Planta Med.* **75**, 1549-1560.
18. Lim, H. J., Jang, H. J., Kim, M. H., Lee, S. Y., Lee, S. W., Lee, S. J. and Rho, M. C. 2019. Oleanolic acid acetate exerts anti-inflammatory activity via IKKα/β suppression in TLR3-mediated NF-κB activation. *Molecules* **24**, 4002.
19. Miura, A., Ishizuka, T., Kanoh, Y., Ishizawa, M., Itaya, S., Kimura, M., Kajita, K. and Yasuda, K. 1999. Effect of tumor necrosis factor-alpha on insulin signal transduction in rat adipocytes: relation to PKC beta and zeta translocation. *Biochim Biophys Acta* **1449**, 227-238.
20. Muhammad, R. S., Ishtiaq, Syed, M. H., Solomon, H., Armando, Z., Akhtar, M., Simona, C., and Inamulllah, K. 2016. Protein tyrosine phosphatase 1B inhibitors isolated from *Artemisia roxburghiana*. *J. Enzyme Inhib. Med. Chem.* **31**, 563-567.
21. Na, M. K., Kim, B. Y., Osada, H. and Ahn, J. S. 2009. Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from *Sorbus commixta*. *J. Enzyme Inhib. Med. Chem.* **24**, 1056-1059.
22. Nazaruk, J. and Borzym-Kluczyk, M. 2015. The role of triterpenes in the management of diabetes mellitus and its complications. *Phytochem Rev* **14**, 675-690.
23. Picardi, P. K., Caricilli, A. M., Abreu, L. L., Campello Carvalheira J. B., Velloso, L. A. and Mário J. A. and Saad, M. J. 2010. Modulation of hypothalamic PTP1B in the TNF-alpha-induced insulin and leptin resistance. *FEBS Lett.* **584**, 3179-3184.
24. Qatanani, M. and Lazar, M. A. 2007. Mechanisms of obesity- associated insulin resistance: many choices on the menu. *Genes Dev.* **21**, 1443-1455.
25. Rahul Checker, Santosh, K. S., Deepak, S., Raghavendra, S. P, Jayakumar, S., Vineet K., Gautam S., Bharat B. A. and Krishna, B. S. 2012. Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant, is mediated through suppression of NF-κB, AP-1 and NF-AT. *PLoS One* **7**, e31318.

26. Saltiel, A. R. and Kahn, C. R. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799-806.
27. Vieira, M. N., Lyra E Silva, N. M., Ferreira, S. T. and De Felice. F. G. 2017. Protein Tyrosine Phosphatase 1B (PTP1B): A potential target for alzheimer's therapy? *Front Aging Neurosci* **9**, 7.
28. Wajant, H. and Siegmund, D. 2019. TNFR1 and TNFR2 in the control of the life and death balance of macrophages. *Front Cell Dev Biol*. **7**, 91.
29. Wilcox, G. 2005. Insulin and insulin resistance. *Clin Biochem Rev* **26**, 19-39.
30. Xiaoan, W., Hongbin, S., Jun, L., Keguang C., Pu, Z., Li, Z., Jia, H., Luyong Z., Peizhou N., Spyros, E. Z., Demetres, D. L., Kyra-Melinda, A., Thanasis G., Joseph, M. H, and Nikos G. O. 2008. Naturally occurring pentacyclic triterpenes as inhibitors of glycogen phosphorylase: synthesis, structure-activity relationships, and x-ray crystallographic studies. *J Med Chem* **51**, 3540-3554.
31. Yang, J., Leng, J., Li, J. J., Tang, J. F., Li, Y., Liu, B. L. and Wen, X. D. 2016. Corosolic acid inhibits adipose tissue inflammation and ameliorates insulin resistance via AMPK activation in high-fat fed mice. *Phytomedicine* **23**, 181-190.
32. Yang, J., Park Y, Zhang H, Xu, X., Glen, A. L., Kevin, C. D. and Zhang, C. 2009. Feed-forward signaling of TNF-alpha and NF-kappaB via IKK-beta pathway contributes to insulin resistance and coronary arteriolar dysfunction in type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* **296**, 1850-1858.
33. Ying, L., Jianwei, W., Tieguang, G., Johji, Y. and Yuhao, L. 2014. Oleanolic acid supplement attenuates liquid fructose-induced adipose tissue insulin resistance through the insulin receptor substrate-1/phosphatidylinositol 3-kinase. *Toxicol Appl Pharmacol* **277**, 155-163.
34. Zhu, K. N., Jiang, C. H., Tian, Y. S., Xiao, N., Wu, Z. F., Ma, Y. L., Lin, Z., Fang, S. Z., Shang, X. L., Liu, K., Zhang, J. and Liu, B. L. 2015. Two triterpeniods from *Cyclocarya paliurus* (*Batal*) *Iljinsk* (*Juglandaceae*) promote glucose uptake in 3T3-L1 adipocytes: The relationship to AMPK activation. *Phytomedicine* **22**, 837-846.

초록 : 3T3-L1 지방세포에서 루페올의 IRS-1의 인산화 조절을 통한 TNF-α 유도 인슐린 저항성 개선 효과

이현아 · 한지숙*
(부산대학교 식품영양학과)

루페올은 5환성 트리테르펜의 일종으로 많은 질병에 치료 효과가 있는 것으로 보고되었으나, 인슐린 저항성에 미치는 영향은 명확하지 않다. 본 연구에서는 3T3-L1 지방세포에서 루페올의 IRS-1 인산화 억제능을 통해 인슐린 저항성 개선효과를 조사하였다. 3T3-L1 세포를 배양하고 TNF-α를 24시간 동안 처리하여 인슐린 저항성을 유도하였다. 서로 다른 농도의 루페올(15, 30 μM) 또는 100 nM의 rosiglitazone을 처리한 세포를 배양한 후, 용해된 세포를 이용하여 western blotting을 시행하였다. 실험결과 루페올은 지방세포에서 TNF-α에 의해 유발되는 인슐린 신호전달의 음성 조절자와 염증 활성화 단백질 kinase에 대한 개선 효과를 나타냈다. 인슐린 신호전달의 음성 조절자인 PTP-1B와 JNK의 활성 및 IKK와 염증활성화 단백질키나아제의 활성을 억제하였다. 또한, 루페올은 IRS-1의 serine 인산화는 하향 조절하고 tyrosine 인산화는 상향 조절하였다. 그 후, 하향 조절된 PI3K/AKT 경로가 활성화되고, GLUT 4의 세포막 전위가 자극되어, 결과적으로 인슐린 저항성이 유도된 3T3-L1 지방세포에서에서 세포내 포도당 흡수가 증가하였다. 본 연구결과, 루페올은 3T3-L1 지방세포에서 인슐린 신호전달 및 염증 활성화 단백질 kinsase들의 음성 조절인자를 억제하여, IRS-1의 serine 인산화를 하향 조절함으로써 TNF-α 유발 인슐린 저항성을 개선할 수 있을 것으로 사료된다.