Thesis of Master

Effect of Dieckol from *Ecklonia Cava* on glucose uptake in muscle cells and fat accumulation in liver cells

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감태로부터 분리된 dieckol이 근육세포 포도당수송 과 간세포 지질축적에 미치는 영향

지도교수 이 대 호

박 덕 배

관 건

이 논문을 의학 석사학위 논문으로 제출함

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Effect of Dieckol from *Ecklonia Cava* on glucose uptake in muscle cells and fat accumulation in liver cells

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Data approved:

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Abstract

Type 2 diabetes is one of the fastest growing public health problems worldwide resulting from both environmental and genetic factors. It is a metabolic disorder that is characterized by hyperglycemia and a number of complications occurred in different organs and tissues. *Ecklonia cava* is a kind of brown sea algae and its extract has known to be anti-adipogenic, anti-inflammatory, and so on. However, less has been known about the action mechanism in regulating glucose and lipid metabolism. The present study investigated the effect of polyphenol fraction of *Ecklonia cava* (ECP) and dieckol (DEK) further isolated from *Ecklonia Cava* on the glucose and lipid metabolism in skeletal muscle cells and liver cells, respectively.

ECP and DEK stimulated glucose uptake as well as the translocation of glucose transporter 4 (Glut4) from cytosol to plasma membrane in L6 muscle cells. ECP and DEK also stimulated PI3 kinase (PI3K)-dependent Akt activity, one of crucial signaling cascades for glucose uptake. Inhibition of PI3K could diminish ECP- and DEK-stimuated glucose uptake and Glut4 translocation. However, ECP and DEK failed to stimulate AMPK. In HepG2 liver cells, ECP and DEK suppressed free fatty acid (FFA)-induced intracellular fat accumulation. AMPK activity was stimulated by DEK.

Taken together, ECP and DEK stimulate glucose uptake independent of insulin in skeletal muscle cells through PI3K-Akt pathway, and suppress fat accumulation in liver cells, respectively. From these results, ECP and DEK have a potential to prevent or treat metabolic disorders like as diabetes, nonalcoholic fatty liver diseases and so on.

Contents

Introduction

Type 2 diabetes, called non-insulin-dependent diabetes mellitus, is a metabolic disorder that is characterized by high glucose in the context of insulin resistance or relative insulin deficiency. Diabetes mellitus can cause many complications, including acute (ketoacidosis, *etc*.) as well as serious long-term (cardiovascular disease, chronic renal failure, retinal damage, nerve damage, etc.) problems. The insulin resistance is a physiological condition where the natural hormone insulin becomes less effective at lowering blood sugars. These lead to increasing in blood glucose levels and cause adverse health effects, depending on dietary conditions. Certain cell types such as fat and muscle cells require insulin to absorb glucose. When these cells fail to respond adequately to circulating insulin, blood glucose levels rise. The liver and muscle regulate glucose levels by absorbing the glucose in the presence of insulin.

Insulin signaling is mediated by a complex network linking to a variety of different processes. Briefly, in the presence of insulin, the insulin receptor phosphorylates insulin receptor substrate (IRS) proteins, which are linked to the phosphatidylinositol 3 kinase (PI3K)-dependent pathway, at present, known as an essential role in insulin-stimulated translocation of glucose transporter 4 (Glut4) from cytosol to plasma membrane. A number of studies using various pharmacological inhibitors, microinjection of blocking antibodies are all consistent with this conclusion (lsakoff *et al*., 1995).

AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, promotes ATP-producing and inhibits ATP-consuming pathways in various tissues (Minokoshi *et al*., 2004). A well known role of AMPK is the regulation of lipid metabolism; it stimulates oxidation of fatty acids and inhibits their synthesis, and it is also involved in promoting glucose uptake (Russel *et al*., 1999). AMPK seems to be responsible in part for this exercise-induced

glucose uptake (Goodyear *et al.,* 2001). But the mechanisms are unclear. Recent evidences indicate that pharmacological activation of AMPK improves blood glucose homeostasis, lipid profile and blood pressure in insulin-resistant rodents, make this protein kinase a novel therapeutic target in the treatment of type 2 diabetes (Jensen. T E *et al*., 2007).

Many natural products have long been used as therapeutics for diabetes in complementary medicine. As an example, *Curcuma longa* has been used for the treatment of diabetes by ayurvedic physicians in India (Babu and Srinivasan, 1996). A study on curcumin (an active principle of rhizome of *C. longa*) has shown that tetrahydrocurcumin (THC) inhibits the formation of advanced glycation end products in streptozotocin (STZ)-induced diabetic rats (Pari and Murugan, 2007). The therapeutic potential of other polyphenols has been also demonstrated from the studies of an acute or chronic administration of polyphenols (EGCG from green tea, resveratrol from grape skin) to diabetes mellitus SD rats (Chi *et al.,* 2007; Kao *et al*., 2000). The *Ecklonia cava* is a brown alga found in the ocean off Korea and Japan. In Korea, brown algae have long been used as a food remedy to promote maternal health after her parturition. Recently, several evidences have demonstrated that Ecklonia cava have various biological activities, such as anti-adipogenic (Kim *et al.*, 2010), anti-inflammatory (Kim *et al*., 2010), anti-oxidant (Lee *et al.,* 2010), and anti-bacterial activities (Chio *et al.,* 2010). However precise action mechanism underlying its anti-diabetic effect remains poorly understood. In the present study, we have examined the anti-diabetic effects of compounds from Ecklonia cava in vitro.

The *Ecklonia cava* polyphenol (ECP) fraction and dieckol (DEK) were isolated from *Ecklonia cava*. In order to characterize the metabolic effect of ECP and DEK, we focus on the effects of ECP and DEK on glucose consumption and fat metabolism in muscle and liver cells respectively. We found that ECP and DEK stimulate the phosphorylation of AKT and the translocation of Glut4 to plasma membrane in L6 muscle cells but failed to stimulate AMPK. ECP and DEK also suppressed the accumulation of fat in HepG2 cells and stimulated AMPK activity. Taken together,

ECP and DEK have a potential to prevent or treat metabolic disorders like as diabetes, nonalcoholic fatty liver diseases and so on.

Materials and methods

1. Reagents

L6 myocytes and $HepG_2$ cells were obtained from Korean Cell Line Bank (KCLB, Seoul, Korea). Dulbecco's minimal essential medium (DMEM) was from Sigma (St. Louis, USA) and fetal bovine serum (FBS) was from PAA (Etobicoke Ontario, Canada). Penicillin-streptomycin was from GIBCO (N.Y., USA) and Lactate dehydrogenenase (LDH) cytotoxicity kit was from TAKARA (Otsu, Shiga Japan). Other reagents were from Sigma.

2. Cell culture

2.1. L6 myotube cells

L6 myocytes were grown in DMEM containing 10% fetal FBS, 100 units/ml penicillin and 100 μg/ml streptomycin in an atmosphere of 5% CO_2 at 37 °C. To differentiate into myotubes, L6 myocytes were incubated in DMEM containing 2% horse serum for 3-4 days and used for experiments

2.2. $HepG_2$ cells

HepG2 cells were grown in DMEM containing 10% fetal FBS, 100 units/ml penicillin and 100 μg/ml streptomycin in an atmosphere of 5% CO_2 at 37°C. Confluent cells were washed twice with phosphate-buffered saline (PBS) and further incubated in serum-free DMEM overnight and used for experiments.

3. Measurement of cytotoxicity

3.1. Lactate dehydrogenase (LDH) assay

Lactate dehydrogenase leakage is a means of measuring membrane integrity as a function of the amount of cytoplasmic LDH released from the cytosol into the

medium. LDH activity was determined following the production of NADH during the conversion of lactate to pyruvate (Fernandez *et al*., 2006). Briefly, cell-free culture medium (50 µl) was collected and then incubated with 50 µl of the reaction mixture of cytotoxicity detection reagents (Takara) in a 96-well microwell plate for 30 min at room temperature. The optical density at 490 nm wavelength was then measured by using the ELISA plate reader (Sunrise, TECAN AUSTRIA).

3.2. MTT assay

MTT assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystals which is largely impermeable to cell membrane, thus resulting in its accumulation within healthy cells. Briefly, 500 µl MTT solution (1 mg/ml) was added into each well and cultured for 30 min at 37 ^{o}C. The supernatant was removed and 2-propanol (500 µl) was added to each well. The optical density at 570 nm wavelength was measured by the ELISA plate reader (Sunrise, TECAN, AUTRIA).

3.3. Hoechst 33342 staining

Hoechst 33342 nucleic acid stain is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound specifically to double-stranded DNA. This dye is often used to distinguish condensed nuclei in apoptotic cells. After treatments, L6 myotube cells or $HepG_2$ cells were incubated with Hoechst 33342 (10 µg/ml) for 30 min and photographed with a fluorescent microscope (Olympus IX-51, Japan) equipped with digital camera (DP71, Olympus, Japan).

4. Glucose assay

The cell-free culture medium (5 µl) was collected and then mix with 150 µl glucose assay reagent (ASAN, Korea) in a 96-well plate for 3 min at room temperature. The optical density at 490 nm wavelength was measured with the

ELISA plate reader (Sunrise, TECAN AUSTRIA).

5. Nile Red staining

$HepG_2$ cells were cultured in a 12-well plate. After two washes with PBS, $HepG_2$ cells were stained for 15 min with Nile red solution (200 ng/ml in 2% acetone/PBS). Cells were then washed with PBS to remove excess stain and then photographed with a fluorescent microscope (Olympus IX51, Japan) equipped with digital camera (DP71, Olympus, Japan).

6. Triglyceride (TG) assay

Methods for triglyceride determination generally involve enzymatic or alkaline hydrolysis of triglycerides to glycerol and free fatty acids followed by either chemical or enzymatic measurement of the glycerol released. Treated $HepG_2$ cells were scrapped from culture dishes and displaced to 1.5 ml microcentifuge tube. The tubes were centrifuged at 3,000 rpm for 5 min. The supernatant was discarded and the pellet was with PBS. TG concentration was measured with TG assay reagents (Sigma, Louis USA) by a manufacturer's protocol. The optical density at 540 nm was measured by ELISA plate reader (Sunrise, Tecan, Austria).

7. Western blot analysis

Cells were preincubated in serum-free medium overnight and then treated with various compounds for purposes. Cells were collected by scrapping and washed with D-PBS. Collected cell pellets were lysed in a lysis buffer (RIPA, Millipore, Billerica, USA) supplemented with inhibitors for various proteases and phosphatases (Sigma, Louis, USA) and kept on ice for 15 min. Lysates were centrifuged at 15,000 rpm at 4℃ for 15 min and the supernatant was stored at -20℃ until use. Protein concentration was determined with BCA protein assay reagent (Pierce, USA). Aliquots of the lysates (15 μg protein) were separated on a 4-12% Tris-Bis gel (Invitrogen, Carlsland, USA) and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, USA) with transfer buffer (Invitrogen, Carlsland, USA) . After blocking the nonspecific site with 5% non-fat dry milk (Santa cruz, CA, USA) in TBS-T buffer. The membrane was incubated with specific primary antibodies at 4℃ for overnight. Primary antibodies against p-AMPK were from Millipore (Bedford, USA), and antibodies against phosphor-Akt, phospho-ERK, and Glut4 were from Santa cruz (CA, USA). The membranes were further incubated with secondary antibodies. The bands of immunoactive protein was visualized with western lightning Plus-ECL reageants (Perkin Elmer, MA, USA) and exposed onto a x-ray film.

8. Preparation of membrane fraction

L6 myotube cells grown in 100 mm plates. Cells were scrapped in 500 μl buffer. Collected cells were placed in 1.5 ml tube and mechanically homogenized and kept on ice for 20 min. After centrifugation at 8,000 rpm for 5 min at 4 °C, the supernatant was transferred into a fresh labeled tube. The supernatant was centrifuged again at 100,000g for 1 h at 4 °C and the precipitate was collected (the membrane fraction).

9. Statistical analysis

Student's t-test and one-way ANOVA were used to determined the statistical significance of difference between for a variety of experimental and control groups. P-values less than 0.05 were considered statistically significant.

Results

1. Effects of ECP and DEK on the viability of L6 myotube cells

We examined whether ECP and DEK are cytotoxic by LDH and MTT assay. Exposure of cells to ECP (62.5~500 μg/μl) for 30 hours showed no significant toxic adverse effects compared with the untreated control. Low dose of DEK (6.25~12.5 μM) also has no toxic effects, but high dose of DEK (25~50μM) has (Fig. 1 A B).

2. Both ECP and DEK stimulate glucose uptake in L6 myotube cells

To determine whether ECP or DEK can increase the glucose uptake in L6 myotube cells, we measured the concentration of glucose in culture medium before and after treatments. The differences of medium glucose between two groups were regarded as the amount of glucose transported into cells. Both ECP and DEK induced a dose-dependent increase of glucose consumption (Fig. 2).

3. ECP and DEK activate Akt phosphorylation in L6 myotube cells

We investigated the mechanism underlying the ECP- and DEK-mediated increase of glucose uptake. We measured the degree of phosphorylation of Akt, a PI3K-dependent signaling molecule that is crucial for Glut4-mediated glucose uptake. Paralled with a increase of glucose transport (Fig. 2), ECP and DEK also stimulated phosphorylation of Akt in dose-dependent manners (Fig. 3).

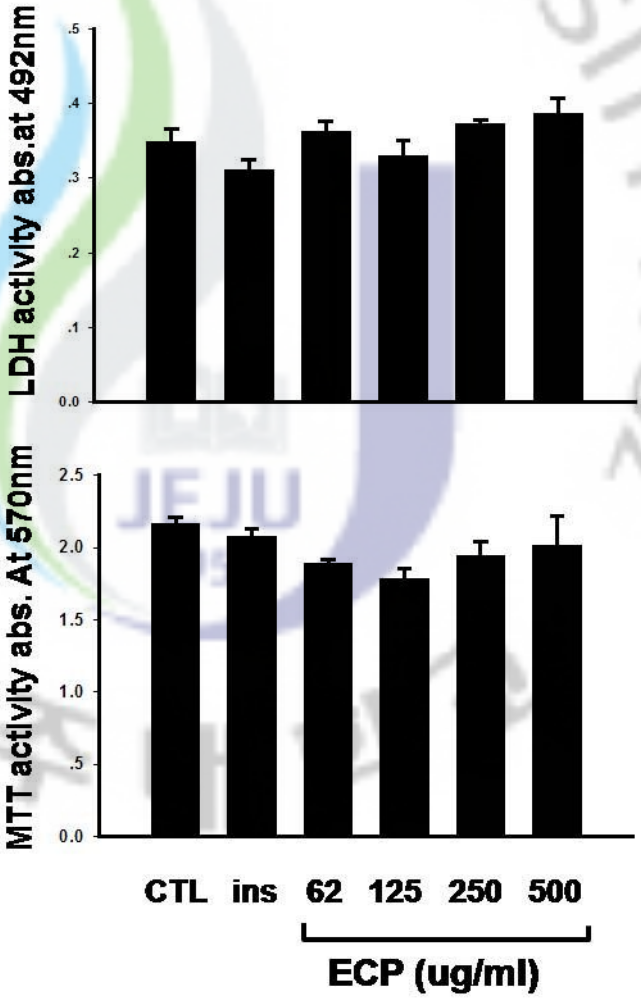

Figure 1A. Effect of ECP on the viability of L6 myotube cells. L6 myotube cells were serum-starved for 4 h and pretreated with four different concentrations of ECP for 30 min before insulin (10 nM) treatment. The cytotoxicity was determined with LDH and MTT assay. Data represent the mean ± SE of triplicate experiments.

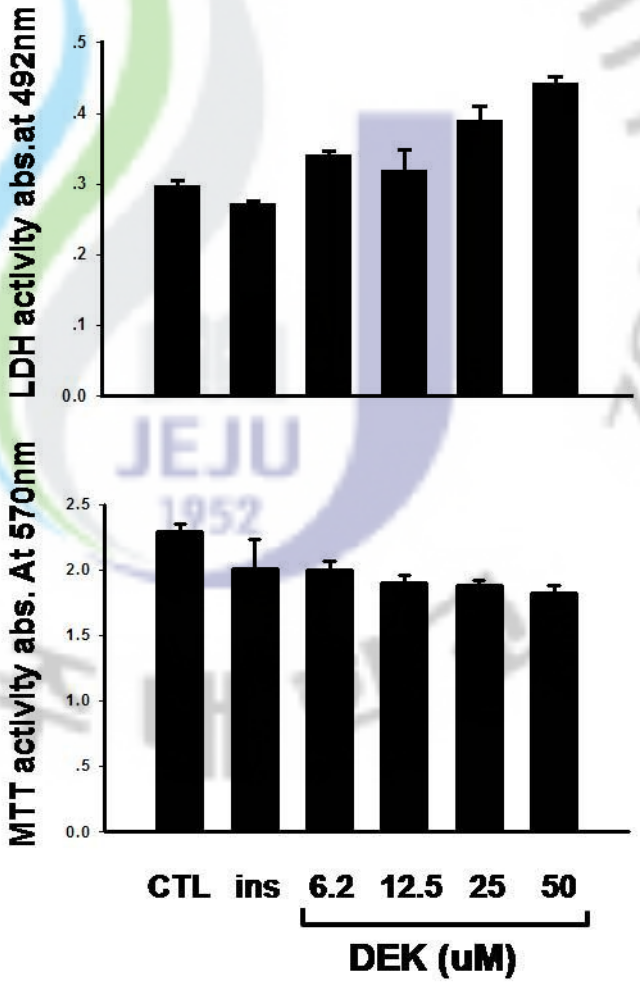

Figure 1B. Effect of DEK on the viability of L6 myotube cells. L6 myotube cells were serum-starved for 4 h and pretreated with four different concentrations of DEK for 30 min before insulin (10 nM) treatment. The cytotoxicity was determined with LDH and MTT assay. Data represent the mean ± SE of triplicate experiments.

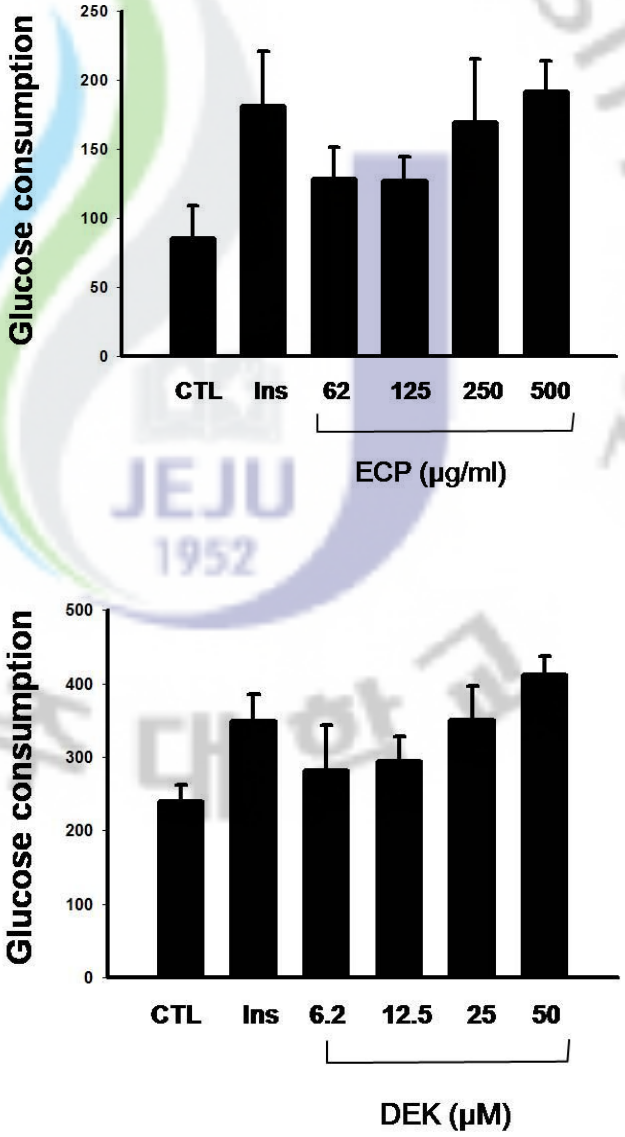

Figure 2. Effects of ECP and DEK on glucose consumption in L6 myotube cells. L6 myotube cells were serum-starved for 4h, pretreated with ECP or DEK for 30 min before insulin (10 nM) treatment. Data represent the mean ± SE of triplicate experiments.

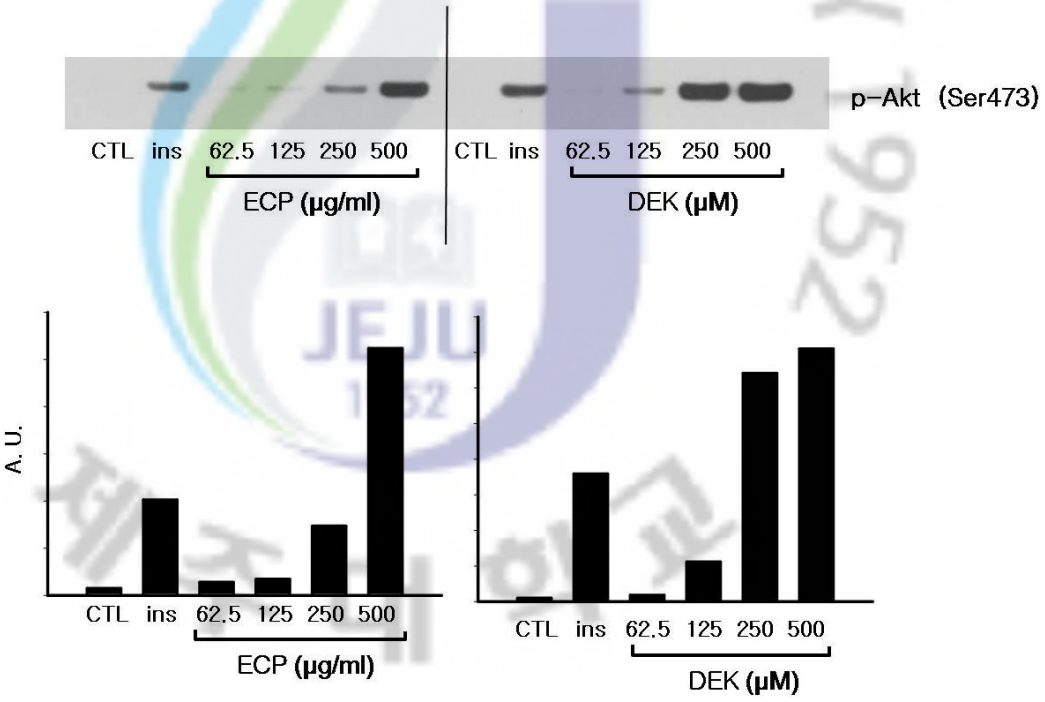

Figure 3. Effects of ECP and DEK on Akt phosphorylation. L6 myotube cells were serum-starved for overnight with D-MEM (4500 mg/L glucose), pretreated with ECP or DEK for 30 min before insulin (10 nM) treatment for 10 min. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'.

4. PI3 kinases but not AMPK is required for ECP- or DEK- induced glucose consumption

To determine whether ECP and DEK increase the glucose consumption through PI3K-Akt pathway and/or AMPK pathway, we pretreated cells with wortmannin, a wellknown PI3K inhibitor, and compound C, a AMPK inhibitor, before treatment with ECP or DEK. Inhibition of PI3K with wortmannin decreased the ECP- or DEK-induced glucose consumption, but inhibition of AMPK with compound C showed no effect on it (Fig. 4). Previous data indicated that ECP and DEK can stimulate Akt phosphorylation. Pretreatment with wortmannin blocked ECP- or DEK-stimulated phosphorylation of Akt (Fig. 5). We also tested if ECP or DEK can stimulate AMPK phosphorylation, however, neither ECP nor DEK can stimulate (Fig.5). These findings suggest that ECP and DEK stimulate glucose uptake through PI3 kinases pathway but not AMPK pathway in L6 myotube cells.

5. ECP and DEK can stimulate Glut 4 translocation in L6 myotube cells

In muscle cells, Glut4 plays a key role in glucose uptake. Thus, we tested whether ECP or DEK can stimulate Glut4 translocation from the cytosol to the plasma membrane. Both ECP and DEK increased the amount of Glut4 in the plasma membrane compared to the control (Fig. 6). In addition, pretreatment of cells with wortmannin decreased the amount of Glut4 in the plasma membrane increased by ECP and DEK (Fig. 7) whereas pretreatment with compound-c could not affect at all (Fig. 8). These results suggest that ECP or DEK can stimulate glucose by PI3K-Akt pathway but not AMPK pathway in L6 myotube cells.

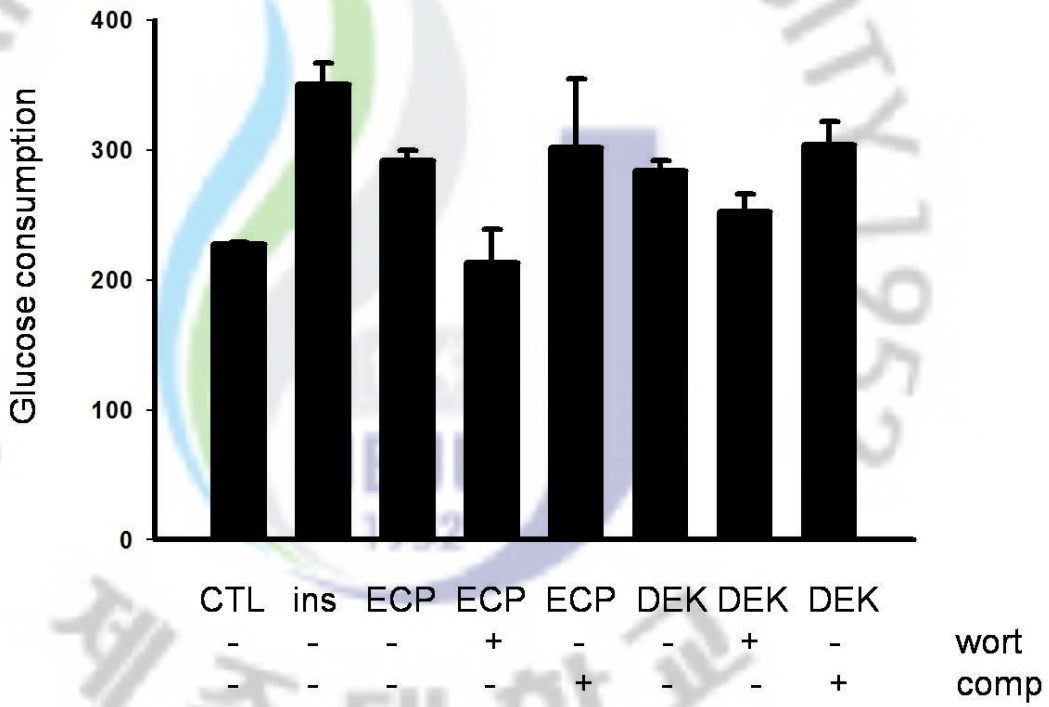

Figure 4. Effects of inhibitors of PI3K and AMPK on ECP- or DEK-induced glucose consumption in L6 myotube cells. L6 myotube cells were serum-starved for 4 h, pretreated with wortmannin (100 μM) or compound C (10 μM) for 30 min, and then treated with ECP (125 μg/ml) or DEK (12.5 μM) for 30 min before insulin (10 nM) addition. Wort, wortmannin; comp, compound C. Data represent the mean ± SE of triplicate experiments.

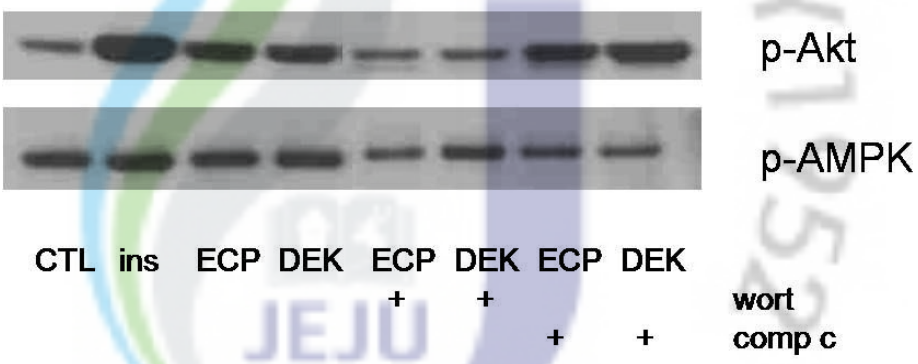

Figure 5. Effects of inhibitors of PI3K and AMPK on ECP- or DEK-induced Akt and AMPK phosphorylation in L6 myotube cells. L6 myotube cells were serum-starved overnight with DMEM (4500 mg/L glucose), pretreated with wortmannin (100 μM) or compound C (10 μM) for 30 min, and then treated with ECP (125 μg/ml) or DEK (12.5 μM) for 30 min before insulin (10 nM) addition. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'. Wort, wortmannin; comp C, compound C.

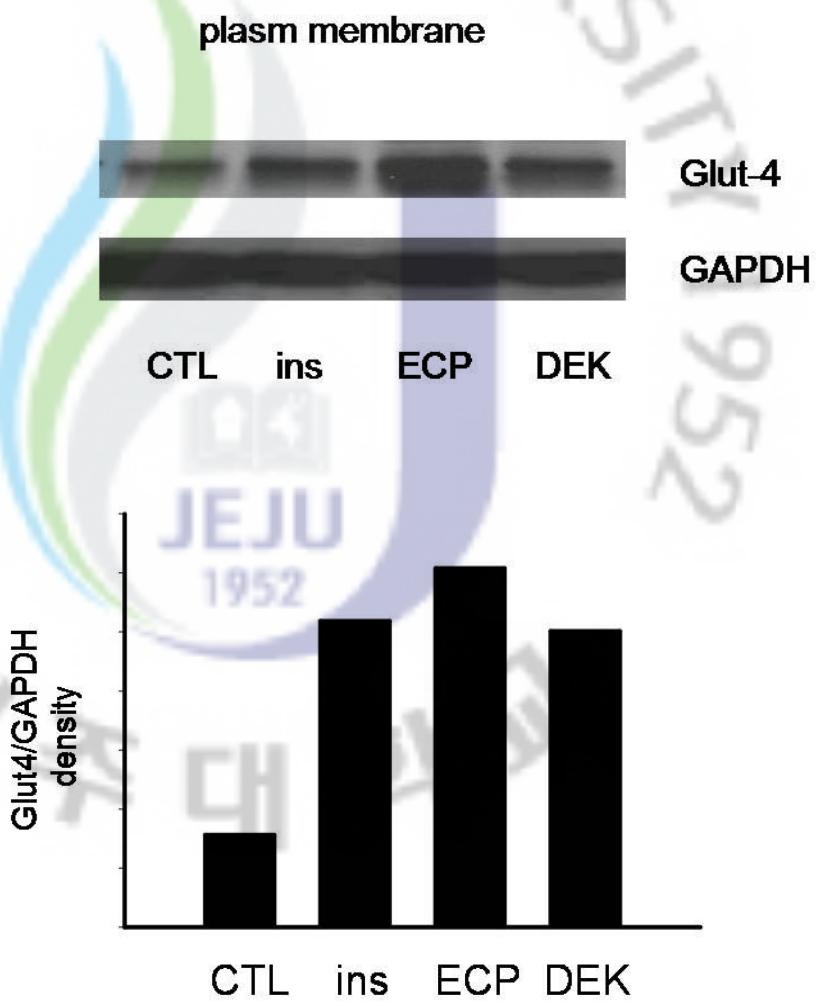

Figure 6. Effects of ECP or DEK on Glut4 translocation in L6 myotube cells. L6 myotube cells were serum-starved overnight with DMEM (4500 mg/L glucose), pretreated with ECP (125 μg/ml) or DEK (12.5 μM) for 30 min before insulin (10 nM) addition. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'.

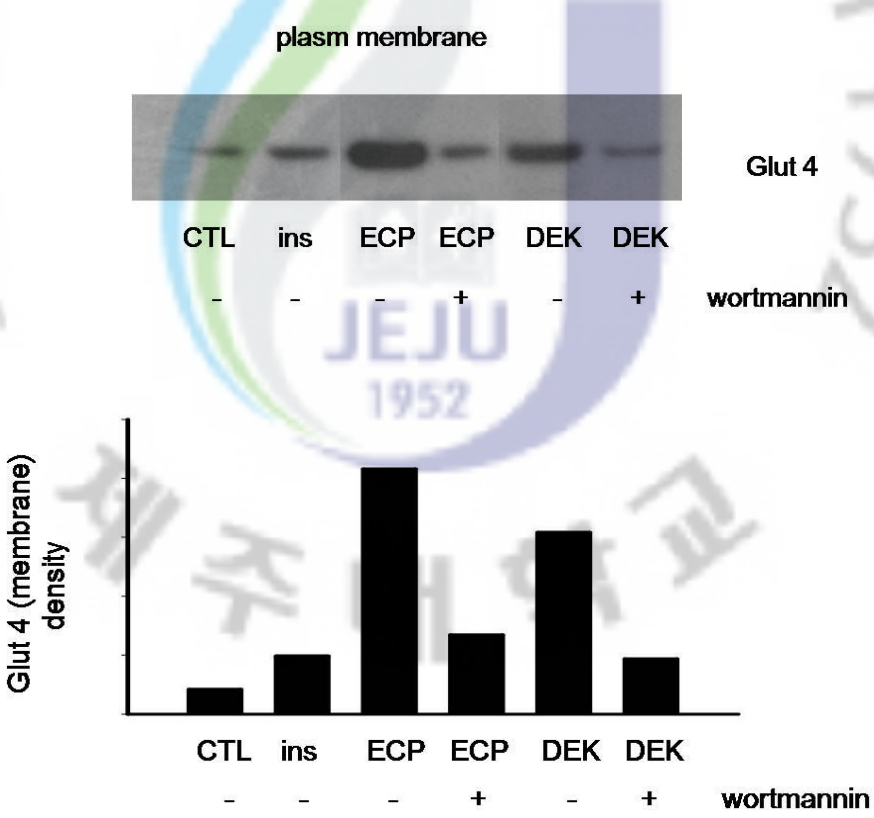

Figure 7. Effects of inhibitors of PI3K on ECP- or DEK-induced Glut4 translocation in L6 myotube cells. L6 myotube cells were serum-starved overnight with DMEM (4500 mg/L glucose), pretreated with wortmannin for 30 min, and then treated with ECP (125μg/ml) or DEK (12.5 μM) for 30 min before insulin addition. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'

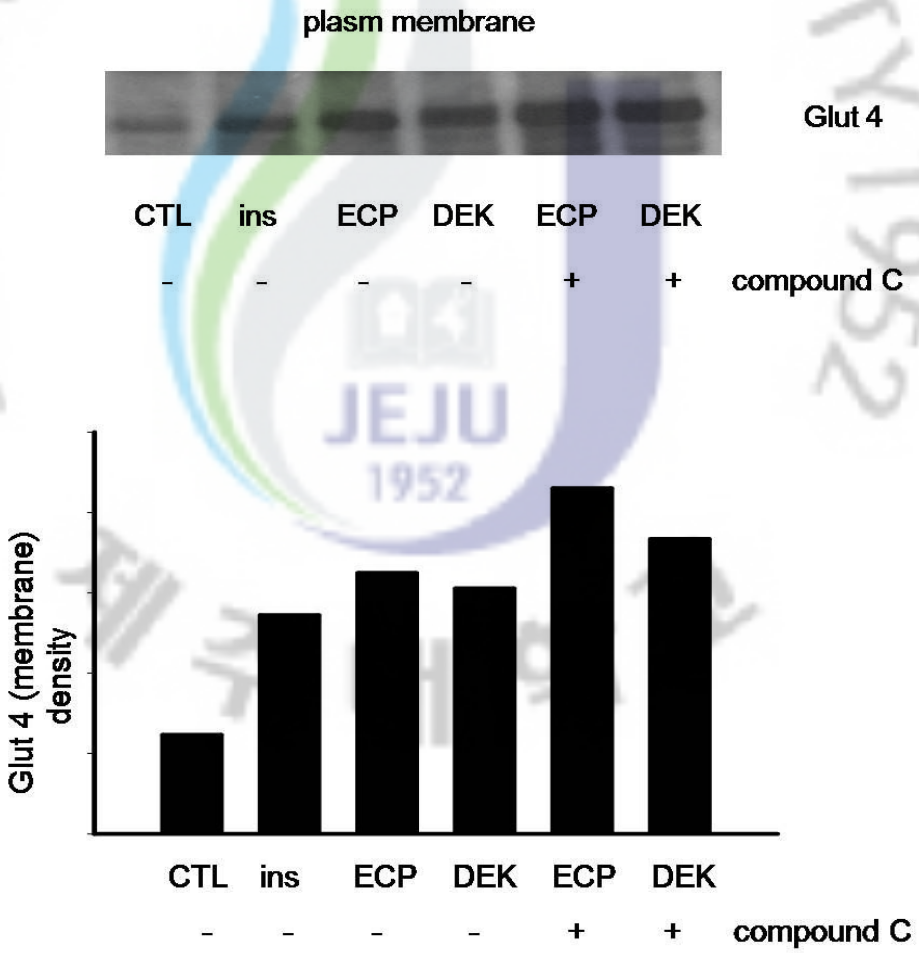

Figure 8. Effects of inhibitors of AMPK on ECP- or DEK-induced Glut4 translocation in L6 myotube cells. L6 myotube cells were serum-starved overnight with DMEM (4500 mg/L glucose), pretreated with compound C for 30 min, and then treated with ECP (125 μg/ml) or DEK (12.5 μM) for 30 min before insulin addition. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'

6. Effects of ECP and DEK on the viability of $HepG_2$ cells

We investigated the effect of ECP and DEK on lipid metabolism in $HepG_2$ liver cells. We examined the cytotoxicity of ECP and DEK in $HepG_2$ liver cells. ECP showed marginal toxic effect on HepG2 cells (Fig. 9A), and DEK did not (Fig. 9B). Same results were also represented from Hoechst 33342 staining experiment (Fig. 10).

7. ECP and DEK suppress fat accumulation in $HepG_2$ cells

We determined whether ECP or DEK affects lipid metabolism in $HepG_2$ cells. $HepG_2$ cells were treated with free fat acid (FFA) to induce intracellular fat accumulation and tested if ECP or DEK can suppress FFA-induced fat accumulation. From the nile red-staining experiments, FFA-induced fat accumulation was suppressed by ECP and DEK (Fig. 11). AICAR is a kind of AMPK activator (positive control) and promotes fat metabolism via stimulating AMPK activity. ECP and DEK also decreased the triglyceride concentration that was increased by FFA treatment (Fig. 12).

8. AMPK is required for DEK-induced lipid metabolism in $HepG_2$ cells

Previous results showed that DEK enhanced lipid metabolism and decrease the triglyceride concentration in the presence of FFA. But the signaling pathway involved in DEK-mediated lipid metabolism is not understood. We investigated the effect of DEK on AMPK phosphorylation. FFA decreased the basal level of AMPK phosphorylation and DEK restored the AMPK phosphorylation decreased by FFA (Fig. 13). These results suggest that AMPK is necessary for DEK-mediated hypolipidemic activity.

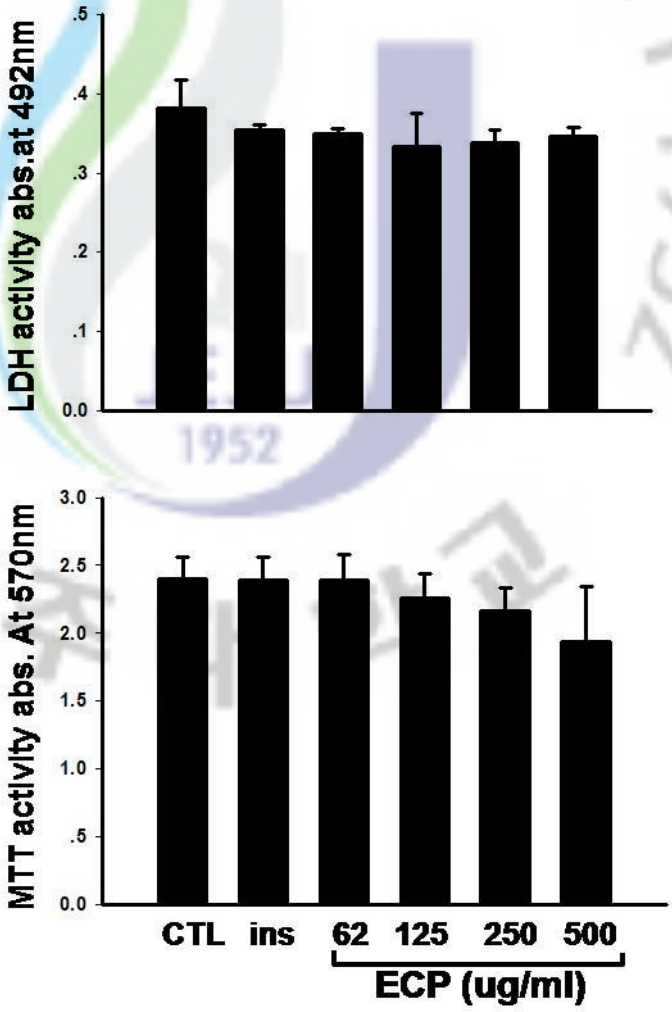

Figure 9A. Effect of ECP on the viability of $HepG_2$ cells. $HepG_2$ cells were serum-starved overnight and pretreated with four different concentrations of ECP for 30 min before insulin (10 nM) treatment. The cytotoxicity were determined with LDH and MTT assay. Data represent the mean ± SE of triplicate experiments.

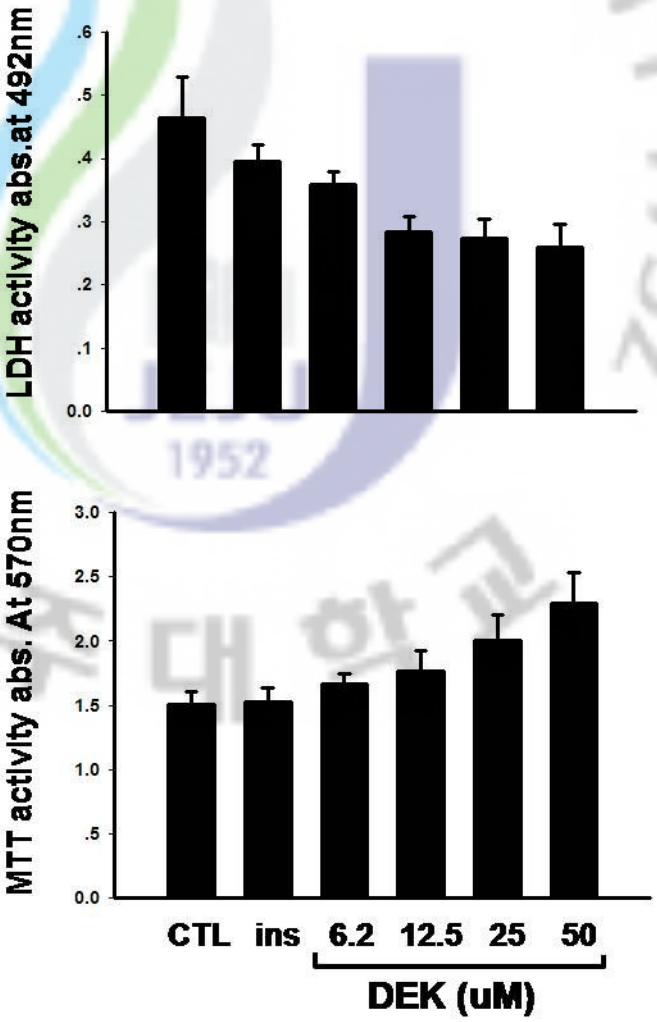

Figure 9B. Effect of DEK on the viability of $HepG_2$ cells. $HepG_2$ cells were serum-starved overnight and pretreated with four different concentrations of DEK for 30 min before insulin (10 nM) treatment. The cytotoxicity were determined with LDH and MTT assay. Data represent the mean ± SE of triplicate experiments.

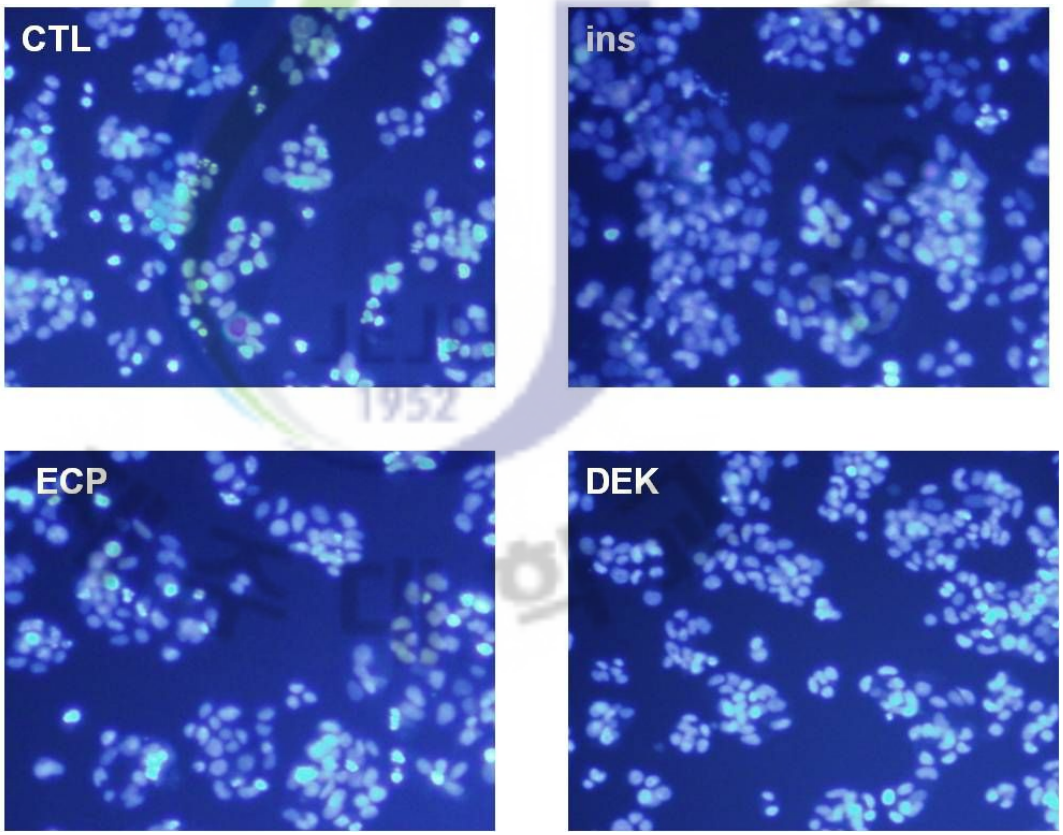

Figure 10. Effects of ECP and DEK on the nuclear condensation in $HepG_2$ cells. $HepG_2$ cells were serum-starved overnight in DMEM and treated with ECP (125 μg/ml) or DEK (12.5 μM). Cultured cells were stained with Hoechst 33342 and photographed as described in 'Materials and Methods'.

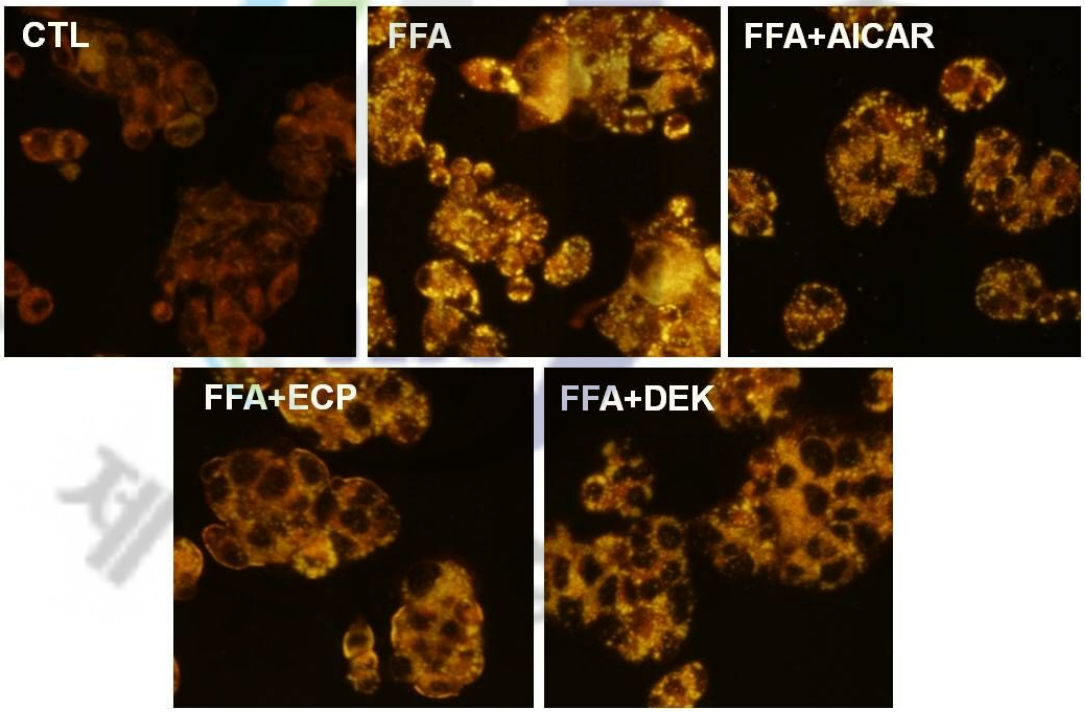

Figure 11. Effects of ECP and DEK on FFA-induced intracellular fat accumulation in HepG$_2$ cells. HepG$_2$ cells were serum-starved overnight in DMEM, pretreated with AICAR (1 mM), ECP (125 μg/ml), DEK (12.5 μM) for 30 min and then treated with FFA (0.1 mM) for 12 h. Cells were stained with nile red as described in 'Materials and Methods'.

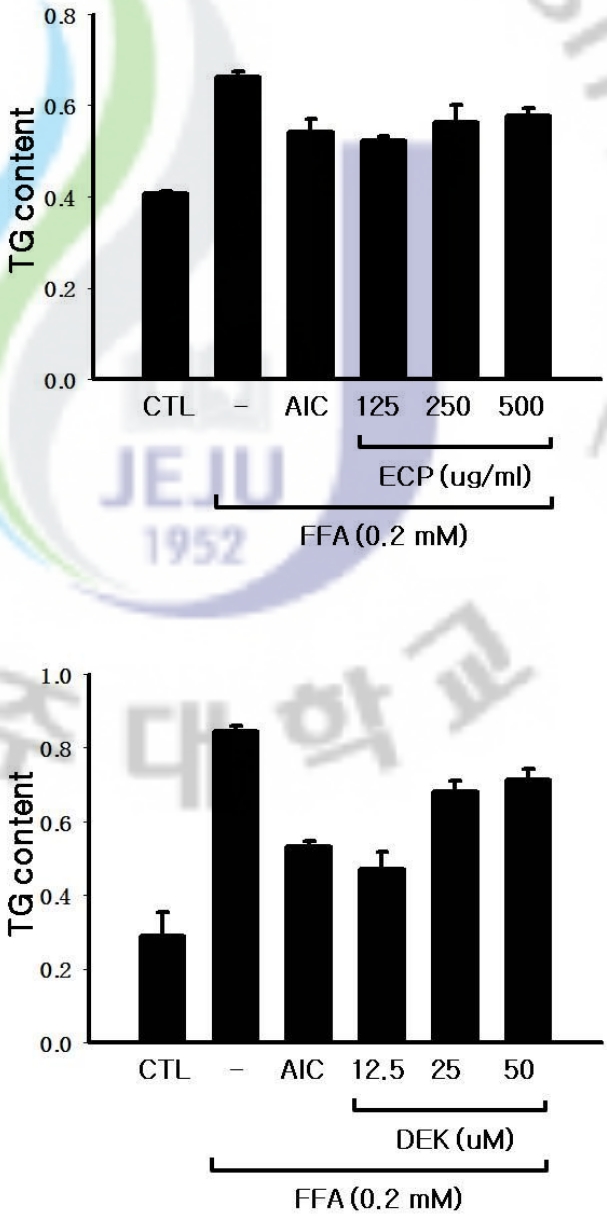

Figure 12. Effects of ECP and DEK on triglyceride accumulation in $HepG_2$ cells. $HepG_2$ cells were serum-starved overnight in DMEM, pretreated with AICAR (1 mM), ECP (125~500 μg/ml), DEK (12.5~50 μM) for 30 min and then treated with FFA (0.2 mM) for 12 h. Data was analysed as described in 'Materials and Methods'.

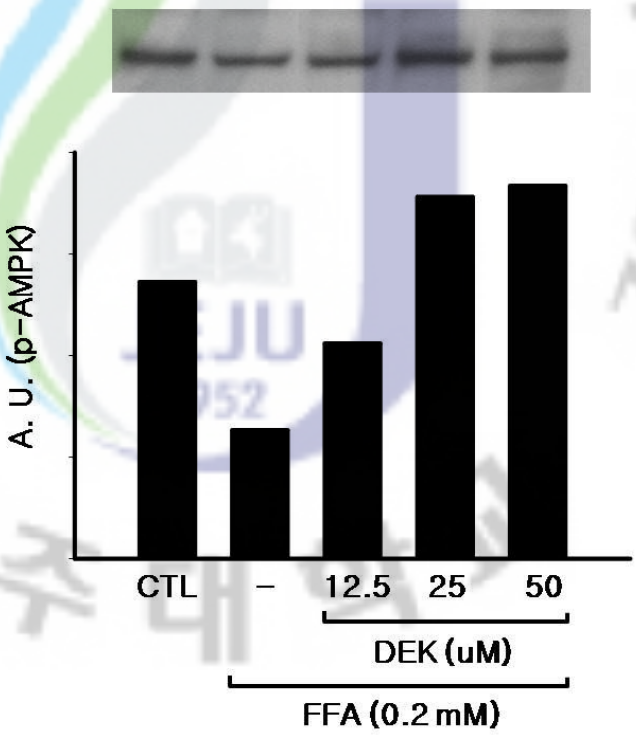

Figure 13. Effect of DEK on AMPK phosphorylation in $HepG_2$ cells. $HepG_2$ cells were serum-starved for overnight with DMEM (4500 mg/L glucose), pretreated with DEK for 30 min. Cells were lysed, separated on SDS-polyacrylamide gels, and immunoblotted as described in 'Materials and Methods'.

Discussion

In the present study, we examined the effects of polyphenol fraction (ECP) of *Ecklonia cava* and dieckol (DEK) on glucose and fat metabolism. The principal findings of our study were that both ECP and DEK stimulate glucose uptake through PI3K-Akt pathway but not AMPK pathway in muscle cells. Both ECP and DEK enhanced the lipid metabolism and DEK might suppress fat accumulation via AMPK in liver cells.

DEK is one compound which is isolated from *Ecklonia cava*. Extract of *Ecklonia cava* contains seven phlorotannin derivatives (eckol, phloroglucinol, fucodiphloroethol G, phlorofucofuroeckol A, dieckol, 7-phloro eckol, and 6,6'-bieckol), along with three common sterols (fucosterol, cholesterol, and ergosterol) (Li *et al.,* 2009). They indicate that dieckol was the strongest antioxidant among the seven phlorotannins of *Ecklonia cava* from various *in vitro* assays. The present study investigated whether DEK can serve to find out a new method to treat various metabolic disorders like as diabetes mellitus and fatty liver. ECP and DEK can stimulate glucose uptake in muscle cells (Fig 2). PI3K-Akt signaling pathway and AMPK pathway play important roles in glucose metabolism. ECP and DEK stimulated Akt phosphorylation and this was inhibited by inhibition of PI3K (Fig 3, 4, 5) in muscle cells. These results suggest that ECP and DEK increase the glucose uptake via PI3 kinase pathway in muscle cells. We also tried to find out the association between ECP and DEK with AMPK pathway in the course of glucose uptake, but no relationship was found. We also determined whether ECP and DEK can increase lipid metabolism in liver cells. The hypolipidemic effect of ECP could not recover the FFA-induced suppression of AMPK phosphorylation (data not shown). In other studies, ECP activates both AMPK/ACC and PI3K/Akt signaling in C_2C_{12} skeletal muscle cells and ECP can recover the diabetic pancreatic function (Kang *et al.,* 2010). These mean that the ECP has a therapeutic potential on type 1 diabetes mellitus. In our study, DEK also has a therapeutic potential on disorders in

the glucose- or lipid metabolism. Activity of DEK is stronger than ECP, even at more lower doses than ECP, suggesting a favorable potential to prevent or treat metabolic disorders.

Taken together, our study showed that both ECP and DEK can increase glucose uptake in muscle cells via PI3 kinase pathway but not AMPK pathway. ECP and DEK also enhance the lipid metabolism in liver cells and AMPK might play a key role in DEK-induced fat metabolism.

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