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Fucoidan Stimulates Glucose Uptake via the PI3K/AMPK Pathway and Increases Insulin Sensitivity in 3T3-L1 Adipocytes

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Brown seaweeds have been shown to decrease blood glucose levels and improve insulin sensitivity previously. In this study, we investigated the effect of fucoidan, a complex polysaccharide derived from brown seaweeds, on glucose uptake to improve insulin resistance, and examined its mechanism of action in 3T3-L1 adipocytes. We observed that fucoidan significantly increased glucose uptake and it was related to an increased expression of plasma membrane-glucose transporter 4 (PM-GLUT4) in 3T3-L1 adipocytes. Fucoidan treatment increased the activation of phosphatidylinositol-3-kinase (PI3K) and the phosphorylation of insulin receptor substrate 1 ($IRS1_{tyr}$) compared with that of the control cells. Fucoidan also promoted the phosphorylation of Akt and protein kinase C (PKC)-λ/ζ compared to that of the control cells. Moreover, fucoidan significantly upregulated acetyl-CoA-carboxylase (ACC) and adenosine monophosphate‐activated protein kinase (AMPK) phosphorylation. As a result, translocation of GLUT4 was significantly enhanced in 3T3-L1 adipocytes, which significantly promoted glucose uptake via the PI3K/AMPK pathways. The elevation of glucose uptake by fucoidan was blocked by inhibitor of PI3K and inhibitor of AMPK in 3T3-L1 adipocytes. These findings indicate that fucoidan might ameliorate glucose uptake through GLUT4 translocation to the plasma membrane by activating the PI3K/Akt and AMPK pathways in 3T3-L1 adipocytes. Fucoidan is thought to be of high material value to diabetes treatments and functional foods.

Key words : 3T3-L1 adipocytes, fucoidan, glucose uptake, GLUT4, PI3K/Akt

Introduction

Type 2 diabetes mellitus, considered one of the most severe chronic conditions, is characterized by hyperglycemia caused by dysfunction in insulin signaling transduction, which would normally stimulate glucose uptake into muscles and adipocytes [2, 21]. Insulin is an important hormone that regulates the homeostasis of blood glucose to maintain its levels within the normal physiological range. Moreover, it promotes glucose uptake into the skeletal muscle and adipose tissue [7] and alleviates hyperglycemia. In the insulin signaling pathway, a deficiency in insulin leads to increased blood glucose levels, causing type 2 diabetes [27]. Glucose uptake is induced via translocation of glucose transporter type 4 (GLUT4) from the intracellular vesicles to the plasma membrane in an insulin-dependent or insulin-independent manner [14].

In detail, glucose uptake is initiated by insulin binding to the insulin receptor (IR) and phosphorylation of the IR substrate (IRS). This phosphorylation causes the downstream activation of phosphatidylinositol-3-kinase (PI3K) and the subsequent activation of Akt and protein kinase C (PKC)-λ/ζ, leading to GLUT4 translocation to the plasma membrane and promotion of glucose uptake into the cells [34]. GLUT4 is an insulin-sensitive glucose transporter; its main role is to mediate insulin-promoted glucose uptake into the cells [19]. Additionally, in an insulin-independent manner, AMP-activated protein kinase (AMPK) plays an important role in promoting glucose uptake by inducing GLUT4 translocation to the plasma membrane [9, 10, 28, 38].

Fucoidan (Fig. 1A), a sulfated polysaccharide derived from *Undaria pinnatifida* and *Fucus vesiculosus*, has potentially beneficial bioactive functions in humans. Fucoidan from *Undaria pinnatifida* contains 68.37% carbohydrates, 21% sulfates, and 10.89% uronic acid [32]. The typical chemical structure of fucoidan includes an L-fucopyranose backbone and alternately connected α (1→3) and α (1→4) linkages [1]. Previous studies have reported the bioactive effects of fucoidan, including its antitumor/anti-cancer [3, 17, 22, 24, 35],

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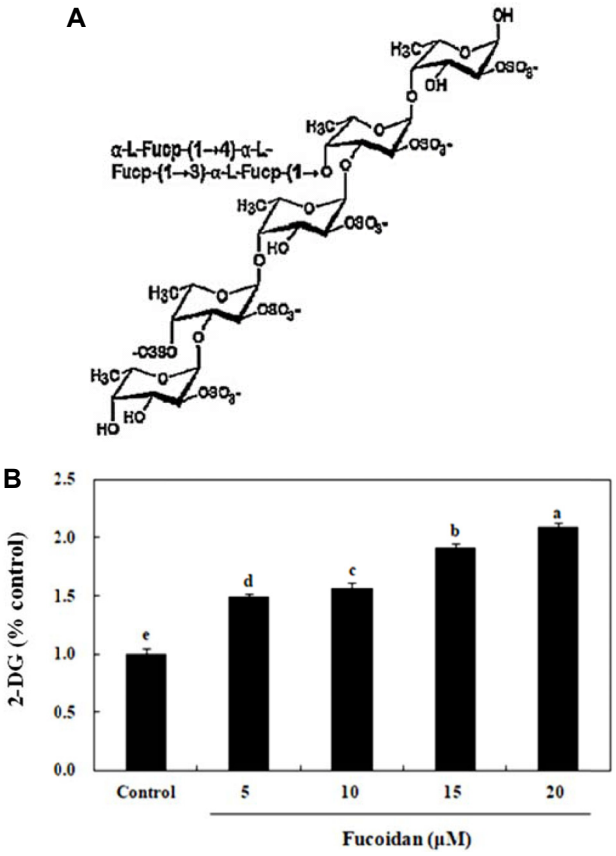

Fig. 1. Typical structure of fucoidan and effect of fucoidan on glucose uptake in 3T3-L1 adipocytes. (A) Structure of fucoidan. (B) The 3T3-L1 adipocytes were treated with 5, 10, 15, and 20 μM of fucoidan for 24 hr prior to the glucose uptake assay. Data are presented as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-e}$values with different superscript letters are significantly dissimilar (p<0.05).

antioxidant [12, 20], anti-inflammatory [30], and anti-coagulant [20] properties. Moreover, Sim et al. [25] reported the effects of fucoidan on lipid accumulation, adipocyte differentiation, lipolysis, and glucose uptake in adipocytes. However, to our knowledge, the effect of fucoidan on glucose uptake and its mechanism has not yet been demonstrated. Therefore, this study aimed to validate the effects of fucoidan on glucose uptake in 3T3-L1 adipocytes by the activation of the insulin signaling pathway.

Materials and Methods

Materials

Fucoidan from *Undaria pinnatifida* was purchased from Sigma-Aldrich (St. Louis, MO, USA; purity ≥95%). Mouse 3T3-L1 pre-adipocyte cells were purchased from the Korean Cell Line Bank (Seoul, Korea). Dulbecco modified Eagle medium (DMEM), fetal bovine serum (FBS), bovine calf serum, wortmannin, and compound C 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, CA, USA). Antibodies against IRS-1, PI3K, phospho-Akt, Akt, and GLUT4 were purchased from Abcam (Cambridge, UK). Antibodies against phospho-IRS-1 were purchased from Thermo Fisher Scientific (Rockford, IL, USA). All chemicals were of analytical grade and were used without any further purification.

Cell culture and adipocyte differentiation

The 3T3-L1 pre-adipocyte cells (Korea Cell Line Bank, Seoul) were grown in 4.5 mM glucose-containing Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), at 37 in an atmosphere of 5% CO_2 and, then, differentiated into adipocytes [8]. Cells were grown for another 24 hr in 10% FCS DMEM, supplemented with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 μg/ml insulin, until they were fully confluent. Thereafter, the 3T3-L1 cells were maintained in fresh DMEM, containing 10% FBS.

Glucose uptake assay

The glucose uptake assay was performed using the 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2 deoxyglucose (2-NBDG) screening system, with some alterations. The 3T3-L1 adipocytes at a density of 1×10^4 cells/well were cultured with DMEM in 96-well and seeded with fucoidan (5, 10, 15, and 20 μM concentrations) and then the cells were incubated for 24 hr. Cells grown in the same method as above were used to examine how inhibitor treatment affected the glucose uptake. The 3T3-L1 adipocytes were seeded with fucoidan 20 μM, fucoidan 20 μM + compound C 10 μM, or fucoidan 20 μM + wortmannin 20 μM, at a density of 1×10^4 cells/ well in 96-well plates. After incubation for 24 hr, the cells were stimulated with 100 nM insulin for 20 min at 37℃ in Krebs–Ringer phosphate buffer solution. The addition of 80 μM 2-NBDG to each well induced glucose uptake. After 1 hr, 2-NBDG uptake was checked using a multilabel counter (Perkin Elmer, Massachusetts, USA). Excitation and emission wavelengths were set at 485 nm and 535 nm, respectively.

Isolation of plasma membranes from 3T3-L1 adipocytes

The 3T3-L1 adipocytes were homogenized by sonication for 5 min at 3 kHz/130 W (UCD-130^{TM}, Cosmo Bio Co., Tokyo, Japan) in ice-cold HES buffer (250 mM sucrose, 20 mM HEPES, and 2 mM EGTA; pH 7.4). The cells were centrifuged at 700× g for 7 min to eliminate cellular debris and nuclei from the homogenate. The harvested supernatant was further centrifuged at 760× g for 10 min to remove the mitochondria. Subsequently, the supernatant was re-centrifuged at 35,000× g for 60 min, and the resulting pellet was used as the cytosolic fraction. GLUT4 was detected by western blotting of the membrane and cytosol fractions. The concentrations of protein in the cytosolic fraction and membrane pellet were quantified using a BCA protein assay kit.

Western blotting

The 3T3-L1 adipocytes were washed twice with ice-cold phosphate-buffered saline, and the total proteins were extracted from the lysis buffer (RIPA: 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 0.1 mM sodium orthovanadate; pH 7.4). After sonication and further centrifugation at 13,000× g at 4℃ for 30 min, the protein content of the resulting supernatant was measured using the BCA protein test kit. A solvent containing 20 μg of protein was subjected to electrophoresis using 10% SDS-polyacrylamide gel. The separated proteins were transferred electrophoretically to a pure nitrocellulose membrane, blocked with a 5% skimmed milk solution for 1 hr, and incubated with primary antibodies (Abcam, Cambridge, UK; 1:1,000) overnight at 4℃. After washing, the blots were incubated with goat anti-rabbit or goat anti-mouse horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 hr. Antigen-antibody complexes were visualized by using ECL Western blotting detection reagents and detected using the luminosis analyzer LAS-1000 Plus (Fujifilm, Tokyo, Japan). Band densities were determined using an image analyzer (Multi Gauge V3.1, Fujifilm, Valhalla, NY, USA). The densities were normalized to the β-actin chemiluminescence signal for relative total and nuclear protein quantification.

Statistical analysis

Data are presented as the mean ± SD from three independent experiments. Statistical analysis, including analysis of variance, was performed using the SAS 9.1 software (SAS Institute, Cary, NC, USA). Significant differences between data were determined using Duncan's multiple range tests.

Results

Fucoidan stimulates glucose uptake in 3T3-L1 adipocytes

Using a 2-DG uptake assay, we first examined whether fucoidan stimulated glucose uptake in 3T3-L1 adipocytes. Fucoidan at 5-20 μM concentrations significantly stimulated glucose uptake in a dose-dependent manner (Fig. 1B). When the cells were treated with 5, 10, 15, and 20 μM fucoidan, glucose uptake was 1.84-, 1.92-, 2.35-, and 2.57-fold greater, respectively, than that of untreated control cells. These results indicated that fucoidan effectively stimulates glucose uptake in 3T3-L1 adipocytes.

Fucoidan enhances the expression of IRS1tyr and PI3K in 3T3-L1 adipocytes

To identify the mechanism by which fucoidan promotes glucose uptake, we examined the phosphorylation levels of $IRS1_{tyr}$, PI3K, Akt, as well as PKCλ/ζ activation. Fucoidan significantly enhanced the phosphorylation of $IRS1_{tyr}$ and activation of PI3K (Fig. 2). Treatment with 20 μM fucoidan upregulated the activation of $IRS1_{tyr}$ and PI3K to 149% and 155%, respectively, of that in the control 3T3-L1 adipocytes. As shown in Fig. 3, fucoidan also significantly increased the phosphorylation of Akt and PKCλ/ζ. Treatment with 20 μM fucoidan significantly upregulated Akt and PKCλ/ζ phosphorylation to 137% and 136%, respectively, of that in the control 3T3-L1 adipocytes. These results indicated that fucoidan enhances the phosphorylation of $IRS1_{tyr}$, Akt, PKCλ/ζ, and the activation of PI3K in the insulin signaling pathway.

Fucoidan increases the expression of acetyl-CoA-carboxylase (ACC)/5'-AMP-activated kinase (AMPK) in 3T3-L1 adipocytes

To identify the mechanism by which fucoidan promotes glucose uptake, we were also investigated AMPK and ACC phosphorylation. In the AMPK pathway, ACC is an essential downstream effector [36] and AMPK phosphorylation enhances GLUT4 translocation to the plasma membrane [18]. As shown in Fig. 4, fucoidan significantly enhanced the phosphorylation of AMPK and ACC. Treatment with 20 μM

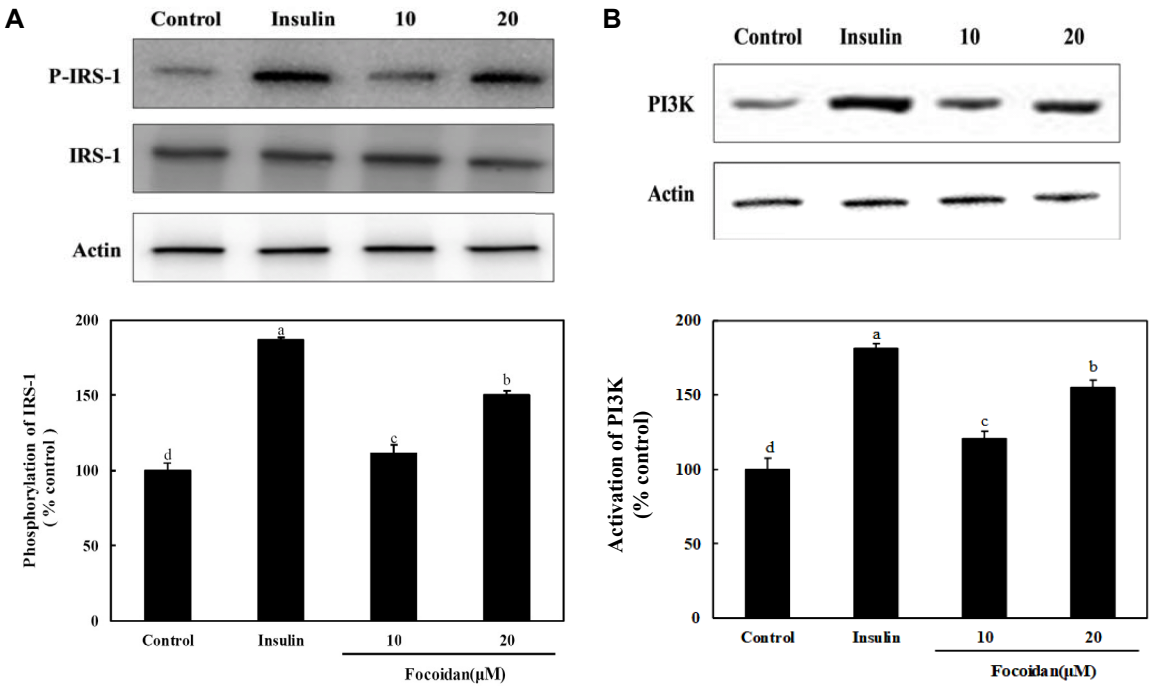

Fig. 2. Effect of fucoidan on expression of IRS-1/PI3K in 3T3-L1 adipocytes. After 24 hr of incubation with 10 or 20 μM fucoidan or 100 nM insulin, the 3T3-L1 adipocytes were lysed and analyzed by immunoblotting. (A) Phosphorylation level of insulin receptor substrate 1 (IRS-1). (B) Activation levels of phosphatidylinositol-3-kinase (PI3K). Data are presented as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-d}$values with different superscript letters are significantly dissimilar (p<0.05).

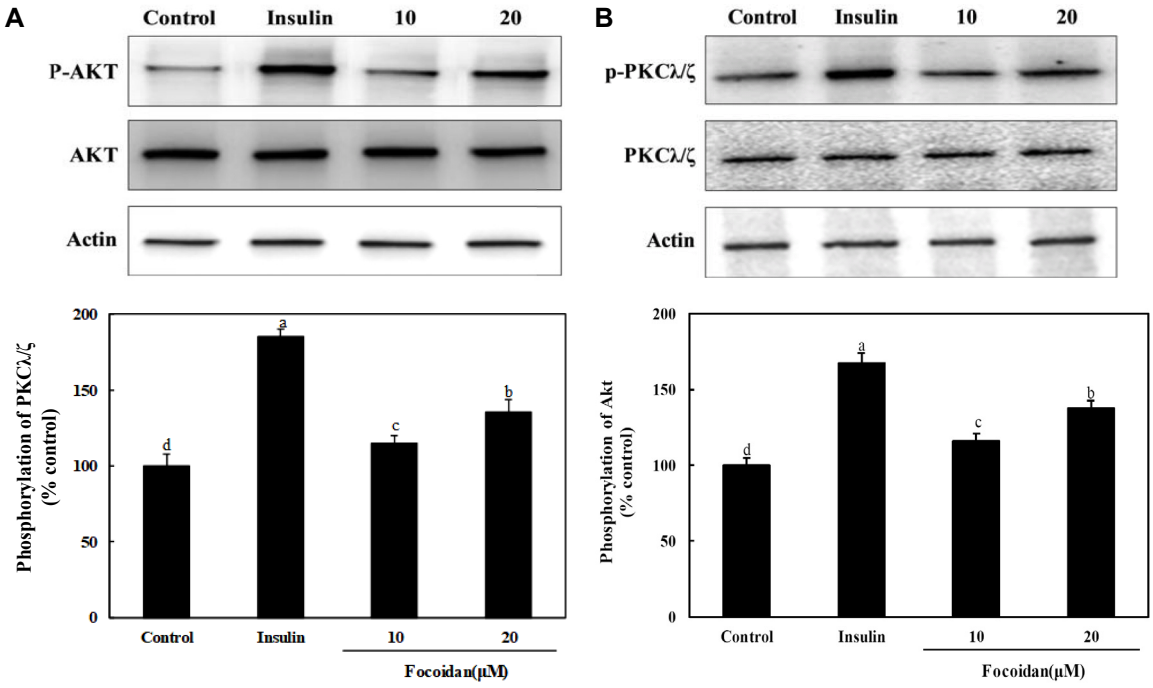

Fig. 3. Effect of fucoidan on expression of Akt/PKC λ/ζ in 3T3-L1 adipocytes. After 24 hr of incubation with 10 or 20 μM fucoidan or 100 nM insulin, the 3T3-L1 adipocytes were lysed and analyzed by immunoblotting. (A) Phosphorylation level of Akt. (B) Phosphorylation level of protein kinase C-λ/ζ (PKCλ/ζ). Data are presented as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-d}$values with different superscript letters are significantly dissimilar (p<0.05).

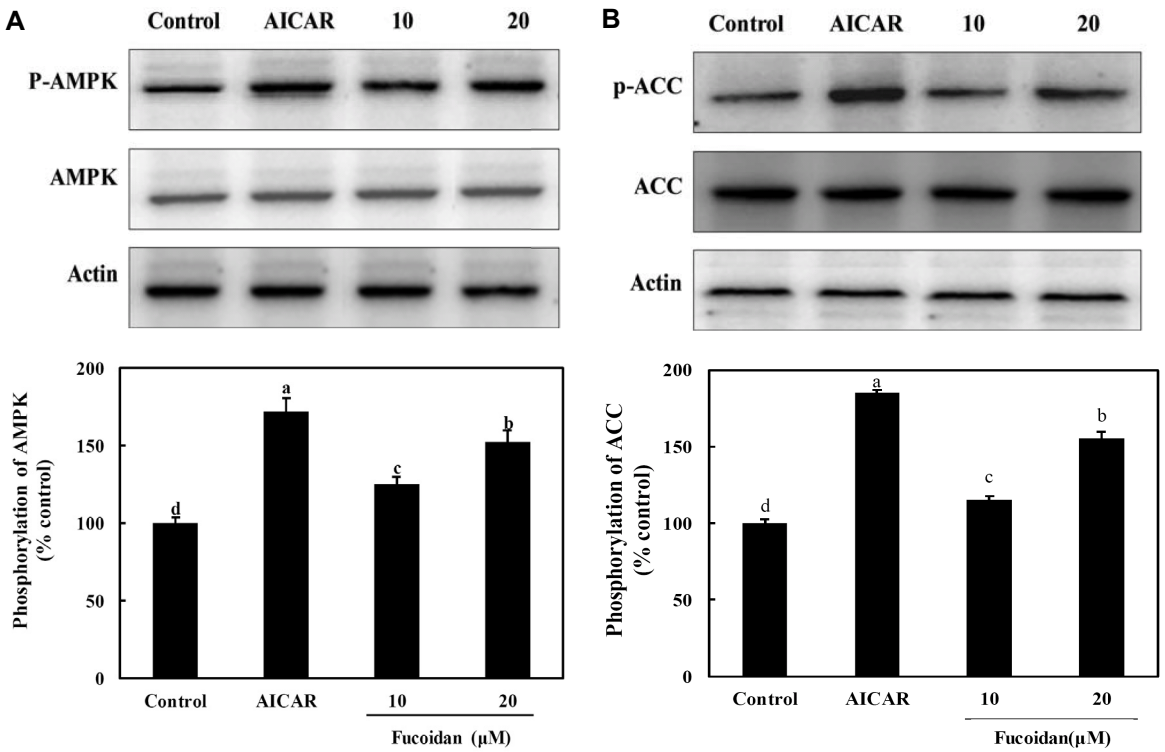

Fig. 4. Effects of fucoidan on expression of AMPK/ACC in 3T3-L1 adipocytes. After 24 hr of incubation with 10 or 20 μM fucoidan or 0.5 mM AICAR(AMPK activator), the 3T3-L1 adipocytes were lysed and analyzed by immunoblotting. (A) Phosphorylation level of AMPK. (B) Phosphorylation level of ACC. Data are presented as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-d}$values with different superscript letters are significantly dissimilar (p<0.05).

fucoidan significantly upregulated the phosphorylation of AMPK and ACC to 152% and 155%, respectively, of that in the control 3T3-L1 adipocytes. These results suggested that fucoidan stimulates glucose uptake by upregulating the phosphorylation of AMPK and ACC.

Fucoidan enhances the expression of plasma membrane GLUT4 (PM-GLUT4)

We examined the expression of PM-GLUT4 to identify the role of GLUT4 in fucoidan-stimulated glucose uptake. As shown in Fig. 5, in 3T3-L1 adipocytes, treatment with 20 μM fucoidan significantly enhanced the expression of PM-GLUT4 to 165% of that in the control. However, treatment with fucoidan, combined with a PI3K inhibitor (wortmannin), downregulated the expression of PM-GLUT4 to 115% of that observed with fucoidan treatment alone. Furthermore, treatment with fucoidan, combined with an AMPK inhibitor (compound C), significantly reduced the expression of PM-GLUT4 to 120% of that observed with fucoidan treatment alone. These results showed that fucoidan upregulated the expression of PM-GLUT4 by activating the PI3K/Akt and AMPK pathways.

Glucose uptake is suppressed by treatment with fucoidan combined with compound C or wortmannin

To confirm the effect of fucoidan, combined with an AMPK inhibitor (compound C) or a PI3K inhibitor (wortmannin), on glucose uptake, we examined glucose uptake in 3T3-L1 adipocytes. As shown in Fig. 6, treatment with fucoidan significantly promoted 2-DG uptake in 3T3-L1 cells. When the cells were treated with 20 μM fucoidan, glucose uptake increased by 2.57-fold compared with the control cells. On the contrary, when the cells were treated with 20 μM fucoidan combined with 20 μM wortmannin or 10 μM compound C, glucose uptake decreased by 1.08- or 1.12-fold, respectively. These results showed that fucoidan can enhance glucose uptake via activating both the PI3K and AMPK signaling pathways.

Discussion

Fucoidan, a sulfated polysaccharide commonly found in

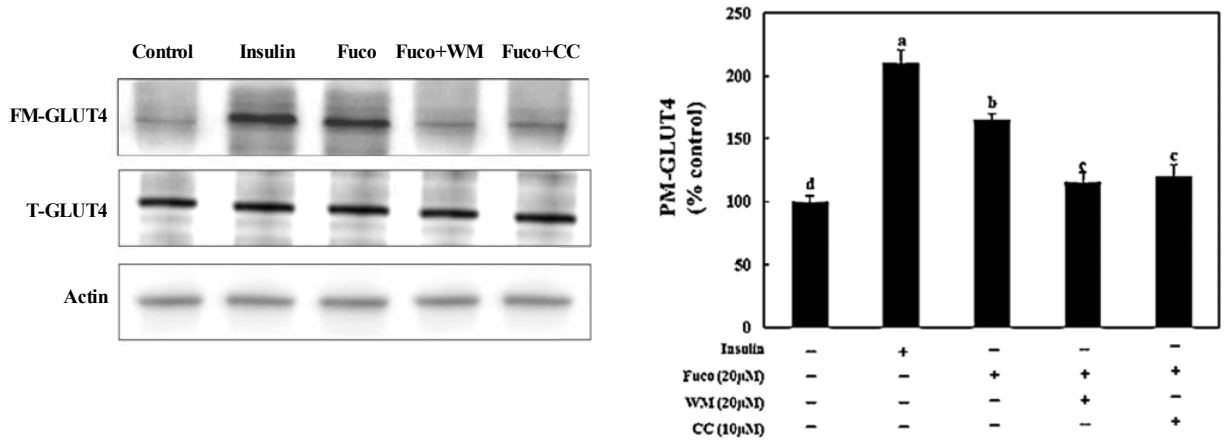

Fig. 5. Effects of fucoidan on expression of PM-GLUT4 protein in 3T3-L1 adipocytes. After 24 hr of incubation with 20 μM fucoidan, 100 nM insulin, 20 μM fucoidan + 10 μM compound C (CC), or 20 μM wortmannin (WM), the 3T3-L1 adipocytes were extracted and analyzed by immunoblotting. Data are presented as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-d}$values with different superscript letters are significantly dissimilar (p<0.05).

various kinds of brown algae, has been widely investigated. It has been reported to exhibit varied biological activities with potential remedial effects. This compound has been found to be effective in preventing diabetes as well as complications related to diabetes [22, 35], although its action on glucose uptake is yet to be demonstrated. In this study, we investigated the effect of fucoidan on glucose uptake in adipocytes and the underlying mechanism mediating this effect. Hyperglycemia, the primary pathological state in type 2 diabetes, occurs when insulin resistance reduces glucose uptake. Thus, enhancing glucose uptake into the cells is an important strategy in reducing hyperglycemia in type 2 diabetes.

Fucoidan significantly promoted glucose uptake into the adipocytes. Promoting glucose uptake into the adipocytes is very crucial in inhibiting the progression of type 2 diabetes and insulin is involved in this process. Adipocytes are the target cells of insulin, and insulin is important for ensuring glucose homeostasis and maintaining physiological levels of blood glucose [15]. Insulin promotes glucose uptake into 3T3-L1 adipocytes by stimulating the translocation of GLUT4 via the PI3K/Akt signaling pathway [23]. In adipocytes, GLUT4 is the major glucose transporter protein, which regu-

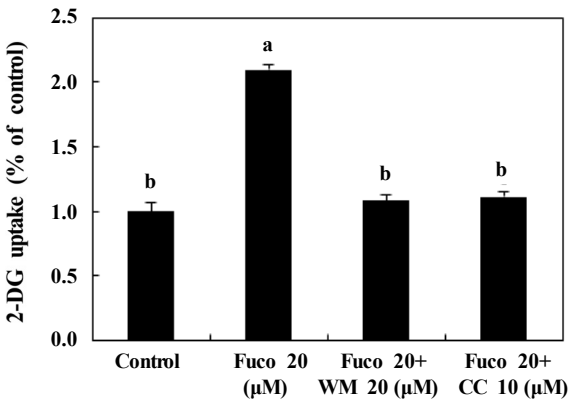

Fig. 6. Effects of fucoidan combined with compound C and wortmannin on glucose uptake in 3T3-L1 adipocytes. The 3T3-L1 adipocytes were incubated with 20 μM fucoidan, 20 μM fucoidan + 10 μM compound C (CC), or 20 μM wortmannin (WM), and then glucose uptake was measured. Data are expressed as mean ± SD of three experiments. Statistical analysis was performed by Duncan's multiple range test, and $^{a-b}$values with different superscript letters are significantly dissimilar (p<0.05).

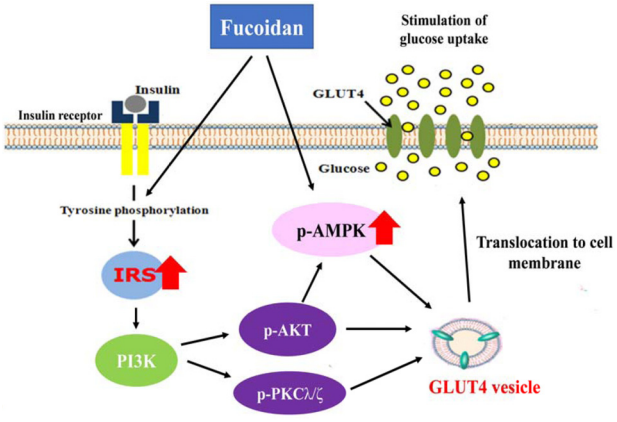

Fig. 7. Proposed mechanism by which fucoidan effects glucose uptake via IRS/PI3K and AMPK signaling. Fucoidan increases glucose uptake as the result of enhanced PM-GLUT4 expression through the activation of the IRS/PI3K and AMPK pathways.

lates glucose uptake and plays an important role in regulating hyperglycemia [6]. GLUT4 translocation to the plasma membrane can be activated via two pathways, namely, the PI3K/Akt and the AMPK pathways. In the PI3K/Akt signaling pathway, insulin is bound to an IR and, then, phosphorylation of $IRS1_{tyr}$ occurs. The combination of $IRS1_{tyr}$ with a regulatory subunit of PI3K via the Src homology 2 (SH2) domain causes PI3K activation, which generates phosphatidylinositol 3,4,5-triphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2) [4]. This insulin-stimulated PI3K signaling pathway diverges into a PKC-mediated pathway and an Akt-dependent pathway [37]. The serine/threonine kinase Akt and atypical PKC isoforms λ and ζ are downstream mediators of PI3K. Their activation induces GLUT4 translocation to the plasma membrane and enhances glucose uptake into the cell.

To identify the underlying mechanism of action involved in the effect of fucoidan on glucose uptake, we examined the activation of $IRS1_{tyr}$/PI3K/Akt and PKCλ/ζ in the insulin signaling pathway. The activation of $IRS1_{tyr}$ and PI3K was significantly upregulated by fucoidan treatment in 3T3-L1 adipocytes; moreover, Akt and PKCλ/ζ phosphorylation was also significantly increased. Phosphorylation of Akt and PKCλ/ζ induced GLUT4 translocation to the plasma membrane, which finally increased PM-GLUT4 expression, thereby promoting glucose uptake. These results suggested that fucoidan can promote glucose uptake via the PI3K/Akt and PKCλ/ζ pathways in 3T3-L1 adipocytes.

Fucoidan, a fucose-containing sulfated polysaccharide that exhibits diverse biological activities, is mainly extracted from brown algae [33]. Its main ingredients are mostly fucose and sulphate with small amounts of galactose, xylose, mannose, and uronic acids. The polysaccharides in fucoidan are polymeric carbohydrate molecules consisting of long chains of monosaccharide units bound by glycosidic linkages. It possesses a backbone built with (1→3)-linked α-L-fucopyranosyl or alternating (1→3)- and (1→4)-linked α-L-fucopyranosyl residues. The promoting effect of glucose uptake by fucoidan in this study is likely due to this structure. Polysaccharides exhibit an anti-diabetic effect by activating the PI3K/Akt/GLUT4 signaling pathway [5, 39]. Recent studies have reported that the structures of these polysaccharides are associated with the enhanced glucose uptake via the insulin signaling pathway. In particular, it was reported that the linkage type, such as (1→3)-linked α-L-fucopyranosyl residues, plays an important role in increasing glucose uptake via insulin signaling [29]. Several studies have reported that fucoidan has a glucose homeostasis effect. Fucoidan regulates postprandial hyperglycemia in normal mice and decreases the levels of blood glucose in type 2 db/db mice [16]. The oral administration of low-molecular-weight fucoidan (LMF) + fucoxanthin decreased blood glucose levels compared with those in the control db/db mice; moreover, treatment of LMF + fucoxanthin was shown to significantly upregulate the expression of IRS-1 and GLUT4 in adipose tissue [26].

Besides the PI3K/AKT signaling pathway, the AMPK signaling pathway is also known to regulate glucose uptake in adipocytes, independently of insulin. AMPK is a conserved intracellular energy sensor that plays a crucial role as a master regulator of intracellular energy homeostasis. AMPK is also an important cellular regulator of glucose metabolism, and it has been considered as a potential treatment target for improving insulin resistance in type 2 diabetes. Metformin, an anti-diabetic drug used by patients with type 2 diabetes, has been reported to enhance glucose uptake via AMPK activation [18]. Activation of AMPK promotes glucose uptake by increasing the translocation of GLUT4 in an insulin-independent pathway [13]. ACC is a necessary effector in the AMPK signaling pathway, and the expression of AMPK induces an increase in ACC phosphorylation [31]. Phosphorylation of these molecules increases glucose uptake and reduces hyperglycemia.

To identify the effect of fucoidan on glucose uptake by AMPK activation, the levels of phosphorylated ACC and AMPK were examined. ACC and AMPK phosphorylation were significantly enhanced by fucoidan treatment in 3T3-L1 adipocytes. An increase in AMPK phosphorylation induces GLUT4 translocation to the plasma membrane, thereby increasing glucose uptake. These results showed that fucoidan could also activate AMPK and ultimately promote glucose uptake. Recent studies have reported that a water-soluble polysaccharide (EPS), produced by *Enterobacter cloacae* Z0206, exhibits a hypoglycemic effect and activates AMPK in KKAy mice. The main component of EPS is a fucose-containing polysaccharide. This component is an important factor affecting AMPK activity [11]. Thus, we strongly suggest that fucoidan, a fucose-containing polysaccharide, might play important roles in glucose uptake via AMPK activation.

In conclusion, fucoidan stimulated glucose uptake into 3T3-L1 adipocytes by activating the PI3K/Akt and AMPK pathways. Activation of these pathways by fucoidan was

confirmed by treatment with the inhibitor of AMPK, compound C, and the inhibitor of PI3K, wortmannin, which decreased the fucoidan-mediated plasma membrane GLUT4 expression and glucose uptake into the cells. Thus, these results suggest that fucoidan could be potentially used as a nutraceutical agent to alleviate hyperglycemia via stimulation of glucose uptake into the cells.

The Conflict of Interest Statement

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

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초록 : 후코이단의 3T3-L1 지방세포에서 PI3K/AMPK 경로를 통한 포도당 흡수 촉진 및 인슐린 민감성 증진 효과

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본 연구는 갈조류 유래 물질인 후코이단이 인슐린 민감성을 증진시키는지를 규명하기 위하여 3T3-L1 지방세포에서 포도당 흡수에 미치는 후코이단의 영향을 측정하고 그 작용기전을 조사하였다. 후코이단은 지방세포에서 포도당 흡수를 유의하게 증가시켰으며 이는 PM-GLUT4의 발현 증가와 관련이 있음을 관찰하였다. 후코이단은 인슐린 신호전달 경로에서 PI3K의 활성화 및 pIRS1tyr, Akt, PKCλ/ζ의 인산화를 대조군에 비해 유의하게 증가시켰다. 또한, AMPK의 활성화를 나타내는 pAMPK 수준이 유의하게 증가하였다. 이들 PI3K 및 AMPK 활성화는 포도당 수송체인 GLUT4를 세포막으로 이동시켰으며 이로 인하여 PM-GLUT4의 발현이 증가되고 포도당 흡수가 촉진되었다. 후코이단에 의한 PI3K 및 AMPK 경로의 활성화를 증명하기 위해, PI3K 억제제인 Wortmannin과 AMPK의 억제제인 Compound C를 사용하여 이들 처리에 의한 포도당 흡수능과 PM-GLUT4의 발현을 측정한 결과 이들의 발현이 유의하게 저해되었다. 따라서 후코이단은 3T3-L1 지방세포에서 PI3K 및 AMPK 경로를 활성화시킴으로써 인슐린 민감성을 증진하고 포도당 흡수를 촉진시킬 수 있음을 나타내었다.