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Pinusolide improves high glucose-induced insulin resistance via activation of AMP-activated protein kinase

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ABSTRACT

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) plays a crucial role in the maintenance of cellular energy homeostasis, and several natural compounds that activate AMPK possibly enhance glucose uptake by muscle cells. In this study, we found that pinusolide stimulated AMPK phosphorylation and glucose uptake and these effects were significantly reduced by siRNA LKB1 or compound C, suggesting that enhanced glucose uptake by pinusolide is predominantly accomplished via an LKB1-mediated AMPK activation pathway. An insulin resistance state was induced by exposing cells to 30 mM glucose, as indicated by reduced insulin-stimulated tyrosine phosphorylation of IRS-1 and glucose uptake. Under these conditions, the phosphorylation of AMPK and ACC were decreased. Surprisingly, disrupted insulin signaling and decreased AMPK activity by high glucose concentrations were prevented by pinusolide. Moreover, this treatment increased insulin-stimulated glucose uptake via AMPK activation. Taken together, our findings suggest a link between high glucose and insulin resistance in muscle cells, and provide further evidence that pinusolide attenuates blockade of insulin signaling by enhancing IRS-1 tyrosine phosphorylation by the activating the AMPK pathway. In addition, this study indicates the targeting of AMPK represents a new therapeutic strategy for hyperglycemia-induced insulin resistance and type 2 diabetes.

1. Introduction

Hyperglycemia is a characteristic feature of type 2 diabetes and plays a pivotal role in diabetes-associated metabolic disorders. Hyperglycemia *per se* induces insulin resistance in experimental animal models, and chronic hyperglycemia plays an important role in perpetuating metabolic deterioration, characteristic of the diabetic state [1]. Thus, hyperglycemia may induce *in vivo* insulin resistance by desensitizing insulin receptors in adipose and muscle tissues [2,3].

AMPK is a phylogenetically conserved intracellular energy sensor and has been implicated in the regulation of food intake, body weight, glucose uptake, and lipid metabolism [4,5]. Once activated, AMPK phosphorylates its downstream substrates to reduce ATP-consuming anabolic pathways responsible for fatty acid, cholesterol, and triacylglycerol synthesis, and increases ATP-generating catabolic pathways leading to fatty acid oxidation and lipolysis [6,7]. Recent reports have shown that AMPK activation may account for at least some of the beneficial effects of exercise, such as, increased fatty acid oxidation [8] and possibly glucose uptake [9]. Furthermore, the administration of the AMPK activator, 5-amino-4-imidazolecarboxamide ribose (AICAR), has been reported to improve glucose tolerance and lipid profiles in insulin-resistant Zucker rats [10]. In addition, the oral biguanide antidiabetic drug metformin improves insulin sensitivity and reduces plasma glucose and lipid levels in patients with type 2 diabetes [11–13]. These observations suggest that AMPK regulates insulin sensitivity and associated hyperglycemia.

The dried leaves of *Biota orientalis* L. (Cupressaceae) have been used in Korean folk medicine to treat gout, rheumatism, and diarrhea, implying that it has anti-inflammatory effects. Furthermore, previous chemical studies on *B. orientalis* have reported a large number of terpenes, flavonoids, and phenolics [14,15], and diterpens, such as, pinusolide which is present in *B. orientalis*, have been reported to have neuroprotective and platelet activating factor (PAF) antagonistic activities [16,17]. However, the effect of pinusolide on insulin resistance and the mechanisms responsible for its action have not been elucidated to date. In the present study, we used L6 myotubes to investigate the effects of pinusolide on glucose uptake and insulin signaling in the presence of high glucose concentrations. We found that the insulin-stimulated tyrosine phosphorylation of IRS-1 and glucose uptake were reduced by high glucose concentrations, but that treatment with pinusolide alleviated high glucose-suppressed insulin signaling by improving the function of IRS-1 molecules. Importantly, the ability of pinusolide to reduce insulin resistance was dampened by siRNA AMPKα2, suggesting that AMPK is required for the hypoglycemic effects of pinusolide.

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2. Materials and methods

2.1. Materials

Pinusolide (Fig. 1A) was isolated from the methanol extract of the leaves of *B. orientalis* and structurally identified as previously described [16]. D-glucose and human recombinant insulin were obtained from Sigma–Aldrich (St. Louis, MO). Fetal bovine serum (FBS), αMEM, trypsin/EDTA, and penicillin/streptomycin were from GIBCO (Auckland, N.Z.), and 2-Deoxy-[^{3}H] D-glucose was from Perkin–Elmer Life Sciences (Boston, MA, USA). Antibodies against phosphospecific and non-phosphospecific-IRS-1, c-Jun NH_2-terminal kinase (JNK), LKB1, AMPK, acetyl CoA carboxylase (ACC), and Akt were purchased from Cell Signaling Technology (Beverly, MA, USA). AMPKα2 polyclonal antibody was purchased from Abcam (Cambridge, MA). All other reagents were of the highest analytical grade commercially available.

2.2. Glucose uptake assay

Radiolabeled 2-Deoxyglucose uptake assays were conducted as previously described [18].

2.3. Cell culture

L6 myotubes were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell culture was performed as previously described [19].

2.4. Immunoblotting and immunoprecipitation with IRS-1

Immunoblot analysis was carried out as previously described [19]. In brief, L6 myotubes were stimulated with reagents or incubated under the indicated conditions. After stimulation, cells were immediately lysed in lysis buffer (20 mM Tris–HCl, pH 8.0, 1% Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 μg/ml pepstatin). Cell debris was removed by centrifugation at 14,000*g* for 15 min at 4 °C, and the resulting supernatant (cell lysate) was used for Western blotting. Protein concentrations were measured using a bicinchoninic acid assay (BCA Protein Assay kit; Pierce). For immunoprecipitation, 1 mg of protein in total cell lysate was incubated with anti-IRS-1 antibody for 2 h at 4 °C and the immunocomplex obtained was precipitated with 20 μl protein A-Sepharose. The precipitate was extensively washed three times with ice-cold lysis buffer. Precipitates or total cell lysates were subjected to 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and resolved proteins were immunoblotted with the indicated antibodies.

2.5. Transfection with small-interfering RNA (siRNA)

For siRNA experiments, SMARTpool for rat LKB1 (ONTARGET*plus* SMARTpool targeting rat LKB1, L-100539-01-0020), and AMPKα2 (L-100623-00-0020) were obtained from Dharmacon (Lafayette, CO). Nonspecific siRNA (ONTARGET*plus* siCONTROL Non-Targeting Pool, D-001810-10-20) was used as a control.

2.6. Statistical analysis

All experiments were performed at least three times. Average values are expressed as means ± SEMs and/or SDs. The analysis was performed using SPSS ver. 9.0 (SPSS, Chicago, IL). The Student's *t*-test was used to compare two independent groups, and statistically significance was accepted for P values of <0.05.

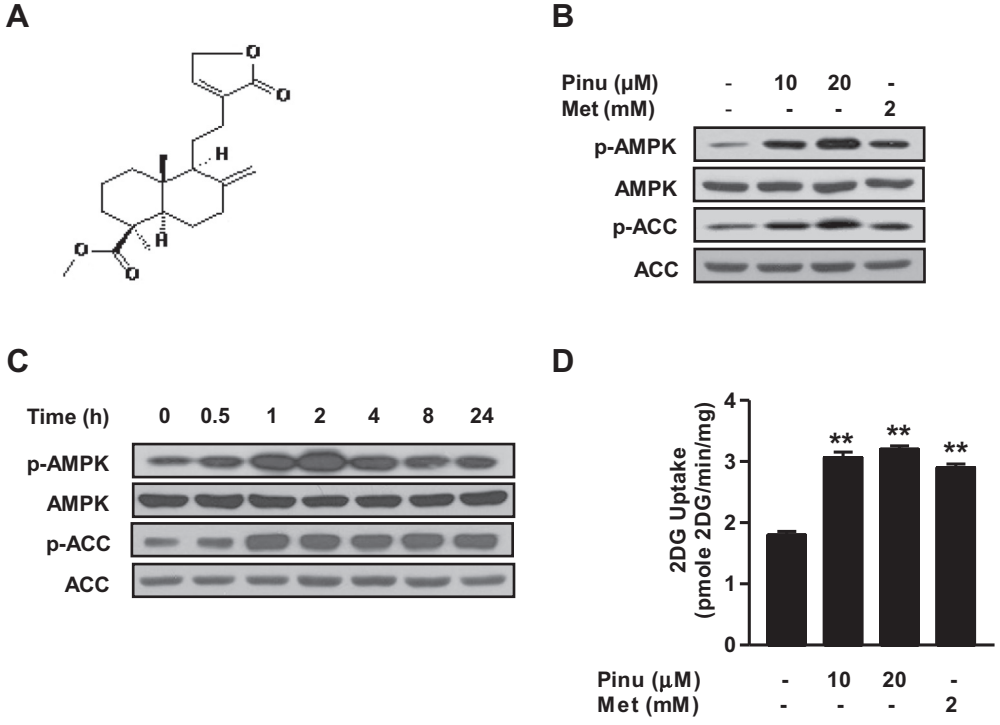

Fig. 1. Pinusolide mediates AMPK activation in L6 myotubes. (A) Chemical structure of pinusolide. (B) L6 myotubes were exposed to different concentrations of pinusolide or metformin (2 mM) for 2 h, and the phosphorylation of AMPK and ACC were then analyzed by Western blotting. Pinusolide was found to increase the phosphorylation of AMPK and ACC dose-dependently. Metforimin was used as a positive control for AMPK phosphorylation. (C) L6 myotubes were treated with pinusolide (10 μM) for various times up to 24 h. The phosphorylations of AMPK and ACC were determined by Western blotting with the indicated antibodies. (D) Cells were serum-deprived and incubated with 10 or 20 μM of pinusolide for 2 h, glucose uptake was then measured. As a positive control, L6 myotubes were treated with 2 mM metformin for 2 h. The glucose uptake shown are representative of five independent experiments. Results are expressed as means ± SEMs (n = 4). $^{**}P < 0.05$ versus untreated controls.

3. Results

3.1. Pinusolide increases the phosphorylation of AMPK and glucose uptake in L6 myotubes

AMPK has been shown to play key roles in the regulations of the metabolisms of glucose and lipids [20]. To examine the cytotoxic effect of pinusolide, L6 myotubes were incubated with various concentrations (10–100 μM) of pinusolide for 24 h. Cells were then incubated for 1 h at 37 °C in a 5% CO_2 atmosphere in the presence of WST-1 (Roche Applied Science, Mannheim, Germany). No cytotoxicity was observed at concentrations up to 100 μM (data not shown). We next examined the effect of pinusolide on the phosphorylation of AMPK and ACC (a target of AMPK). L6 myotubes were treated with or without the indicated concentrations of pinusolide or metformin (a well-known AMPK activator) for 2 h. As shown in Fig. 1B, the levels of AMPK and ACC phosphorylation were considerably increased by pinusolide in a dose-dependent manner, and maximal increases were observed at a pinusolide concentration of 20 μM. As was expected, metformin augmented the phosphorylation of AMPK and ACC. In addition, pinusolide significantly and time-dependently increased the phosphorylation of AMPK and ACC (Fig. 1C).

Accumulating evidence suggests that AMPK activation plays a role in the regulation of skeletal muscle glucose uptake [21,22]. To investigate the effects of pinusolide on glucose uptake, L6 myotubes were incubated in the presence of 10 or 20 μM of pinusolide for 2 h. Glucose uptake increased from 1.8 ± 0.1 pmol/mg/min to 3.1 ± 0.1 pmol/mg/min in pinusolide (10 μM)-treated cells (72% induction; $P < 0.05$) and to 3.2 ± 0.1 pmol/mg/min in pinusolide (20 μM)-treated cells (78% induction; $P < 0.05$) and to 2.9 ± 0.1 pmol/mg/min in metformin-treated L6 myotubes (61% induction; $P < 0.05$), respectively (Fig. 1D). Together, these results demonstrate that pinusolide improves glucose profiles in muscle cells.

3.2. LKB1 is required for pinusolide-induced AMPK activation

To confirm that the increase in glucose uptake by pinusolide was due to its activation of AMPK, we applied an AMPK inhibitor, compound C. As shown in Fig. 2A, pinusolide-mediated the phosphorylation of AMPK and ACC were completely abrogated by pretreating cells with 10 μM of compound C, but total AMPK and ACC protein levels were unchanged. To determine whether the pinusolide-induced increase in glucose uptake was attributable to the activation of AMPK, we treated L6 myotubes with compound C. Glucose uptake was increased about 1.7-fold by 10 μM of pinusolide (3.3 ± 0.1 pmol/mg/min) ($p < 0.05$) versus untreated cells (1.9 ± 0.1 pmol/mg/min) (Fig. 2B). However, when L6 myotubes were pretreated with 10 μM of compound C for 30 min and then incubated with pinusolide, glucose uptake reduced to 40% of that in cells treated with pinusolide alone (3.3 ± 0.1 pmol/mg/min in pinusolide-treated versus 2.0 ± 0.1 pmol/mg/min in compound C-pretreated) (Fig. 2B). Taken together, these results

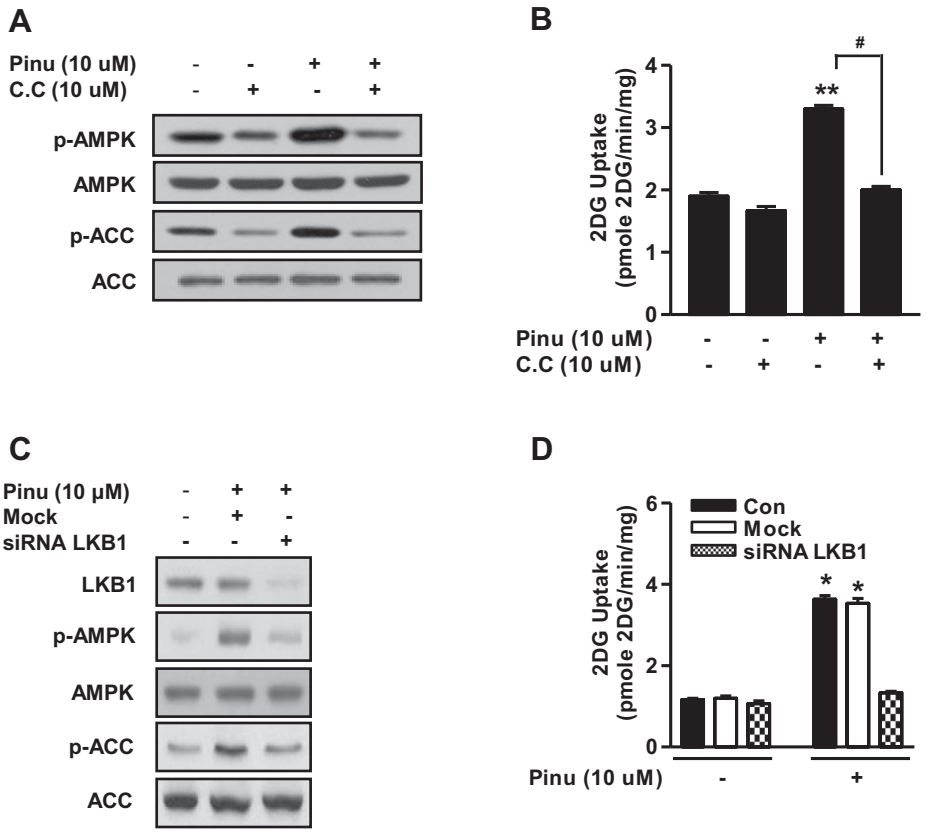

Fig. 2. Specificity of pinusolide in AMPK signaling. (A) Increased phosphorylations of AMPK and ACC in L6 myotubes treated with 10 μM of pinusolide for 2 h were prevented by pretreatment with 10 μM of compound C for 20 min. Phosphorylated AMPK and ACC were immunoblotted with specific antibodies as indicated. (B) Increased glucose uptake after treatment with 10 μM of pinusolide for 2 h was significantly inhibited by pretreating with 10 μM of compound C for 20 min. The results shown are representative of five independent experiments. Results are expressed as means ± SEMs ($n = 4$). $^{**}P < 0.05$ versus untreated controls (Student's t test) and $^{\#}P < 0.05$ versus pinusolide-treated cells (Student's t test or ANOVA). (C) L6 myotubes were transfected with control siRNA (Mock) or LKB1 siRNA for 48 h, and then treated with pinusolide for 2 h. Phosphorylated AMPK and ACC were immunoblotted with specific antibodies as indicated. (D) L6 myotubes were transfected with mock or LKB1 siRNA for 48 h, and then stimulated with or without pinusolide (10 μM) for 2 h. Representative glucose uptake and quantifications of five independent experiments are shown. Results are expressed as means ± SEMs ($n = 4$). $^{*}P < 0.05$ versus untreated controls.

show that pinusolide improved glucose uptake via AMPK activation.

LKB1 is the major upstream kinase of AMPK [23,24] and regulates AMPK activity by phosphorylating its Thr^{172} regulatory site [25,26]. We used siRNA-mediated LKB1 knockdown to explore LKB1 involvement in pinusolide signaling. In L6 myotubes treated with control siRNA, pinusolide robustly increased the phosphorylation of AMPK. In contrast, knockdown of LKB1 blocked the phosphorylation of AMPK by pinusolide (Fig. 2C). Consistent with the lack of AMPK activation in L6 myotubes treated with siRNA LKB1, pinusolide also failed to induce the phosphorylation of ACC (Fig. 2C). In control siRNA transfected L6 myotubes, pinusolide significantly increased glucose uptake, but had no significant effect on glucose uptake in L6 myotubes treated with siRNA LKB1 (Fig. 2D). These findings suggest that LKB1 is essential for pinusolide -stimulated glucose uptake in L6 myotubes.

3.3. Effects of high glucose on the insulin stimulation of the tyrosine phosphorylation of IRS-1 and glucose uptake

To investigate whether high glucose culture impairs insulin signaling, we performed western blot analysis to investigate insulin-stimulated tyrosine phosphorylation of IRS-1 in L6 myotubes. As shown in Fig 3A, exposure of L6 myotubes to glucose (30 mM, 24 h) decreased insulin-stimulated phosphorylation of IRS-1 and Akt without changing their total protein levels. Akt is downstream of PI3K and facilitates insulin-mediated glucose uptake in muscles. In concert with these changes in the phosphorylation of IRS-1 and Akt, insulin-stimulated glucose uptake was significantly decreased by high glucose concentrations (30 mM) in L6 myotubes (Fig. 3B). It has been reported that the activity of c-Jun NH_2-terminal kinase (JNK) is abnormally elevated in various tissues under diabetic conditions [27,28] and that activation of the JNK pathway interferes with insulin action by increasing the serine phosphorylation of IRS-1 [27,29]. As was expected, high glucose increased the phosphorylation of JNK (Fig. 3A). Notably, high glucose concentrations (30 mM) was found to suppress the phosphorylation of AMPK and ACC (Fig. 3A). These results demonstrated that high glucose concentrations suppress AMPK activity and insulin signaling, and thus, inhibit glucose uptake. To determine whether pinusolide improves impaired insulin signaling by high glucose concentrations, L6 myotubes were treated with pinusolide for 2 h under high glucose concentrations. Consistent with our earlier observations, the phosphorylation of AMPK and ACC was markedly increased by pinusolide in the presence of high glucose concentrations (Fig. 3A). Moreover, the inhibition of insulin-mediated the phosphorylation of IRS-1 and Akt and glucose uptake in cells exposed to high glucose concentrations was improved by pinusolide (Fig. 3A and B). Consistent with this observation, JNK phosphorylation, which was increased by high glucose concentrations, was reduced by pinusolide (Fig. 3A), suggesting that pinusolide improves impaired insulin signaling caused by high glucose concentrations in insulin-resistant L6 myotubes.

3.4. Pinusolide improves insulin resistance by activating AMPK

We evaluated whether AMPK activation by pinusolide contributed to the prevention of the reduction of insulin-stimulated tyrosine phosphorylation of IRS-1 and Akt phosphorylation caused by high glucose concentrations in L6 myotubes. Fig. 4A shows that pinusolide did not induce the phosphorylation of AMPK and ACC in AMPKα2, siRNA-transfected L6 myotubes, whereas pinusolide activated AMPK in control siRNA-transfected L6 myotubes. Moreover, high glucose concentrations reduced insulin-stimulated tyrosine phosphorylation of IRS-1 and Akt phosphorylation and this effect was not observed in cells exposed to pinusolide (Fig. 4B).

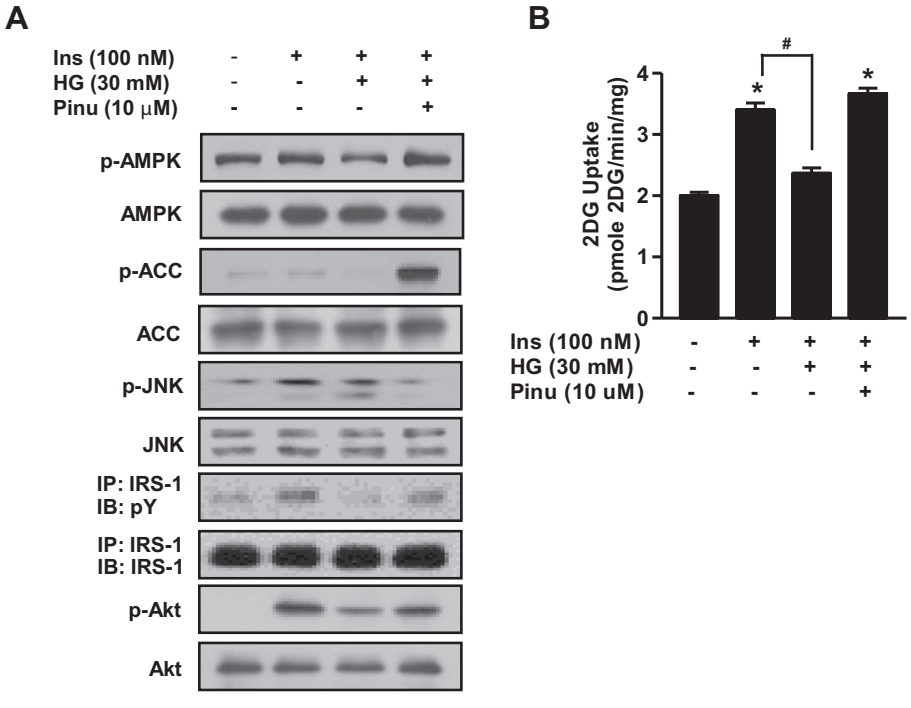

Fig. 3. Pinusolide improves high glucose concentrations-induced insulin resistance in L6 myotubes. (A) L6 myotubes were quiesced in serum-free medium overnight and incubated in serum free medium containing either 5.5 mM (normal) or 30 mM (high concentration) of D-glucose for an additional 24 h, pretreated with or without pinusolide for 2 h, and then stimulated with or without insulin (100 nM) for 10 min. IRS-1 tyrosine phosphorylation (pY), Akt phosphorylation, AMPK phosphorylation, ACC phosphorylation, and JNK phosphorylation and their total protein levels were examined either by immunoprecipitation (IP) following by immunoblotting (IB) or by directly immunoblotting the cell lysates of pinusolide-treated L6 myotubes. (B) L6 myotubes were starved in serum-free medium overnight and incubated in serum free medium containing either 5.5 mM (normal) or 30 mM (high concentration) of D-glucose for 24 hl, pretreated with or without pinusolide for 2 h and then stimulated with or without insulin (100 nM) for 10 min. Representative glucose uptake and quantifications of five independent experiments are shown. Results are expressed as means ± SEMs ($n = 4$). $^{*}P < 0.05$ versus untreated control (Student's t test) and $^{\#}P < 0.05$ versus insulin-treated cells (Student's t test or ANOVA).

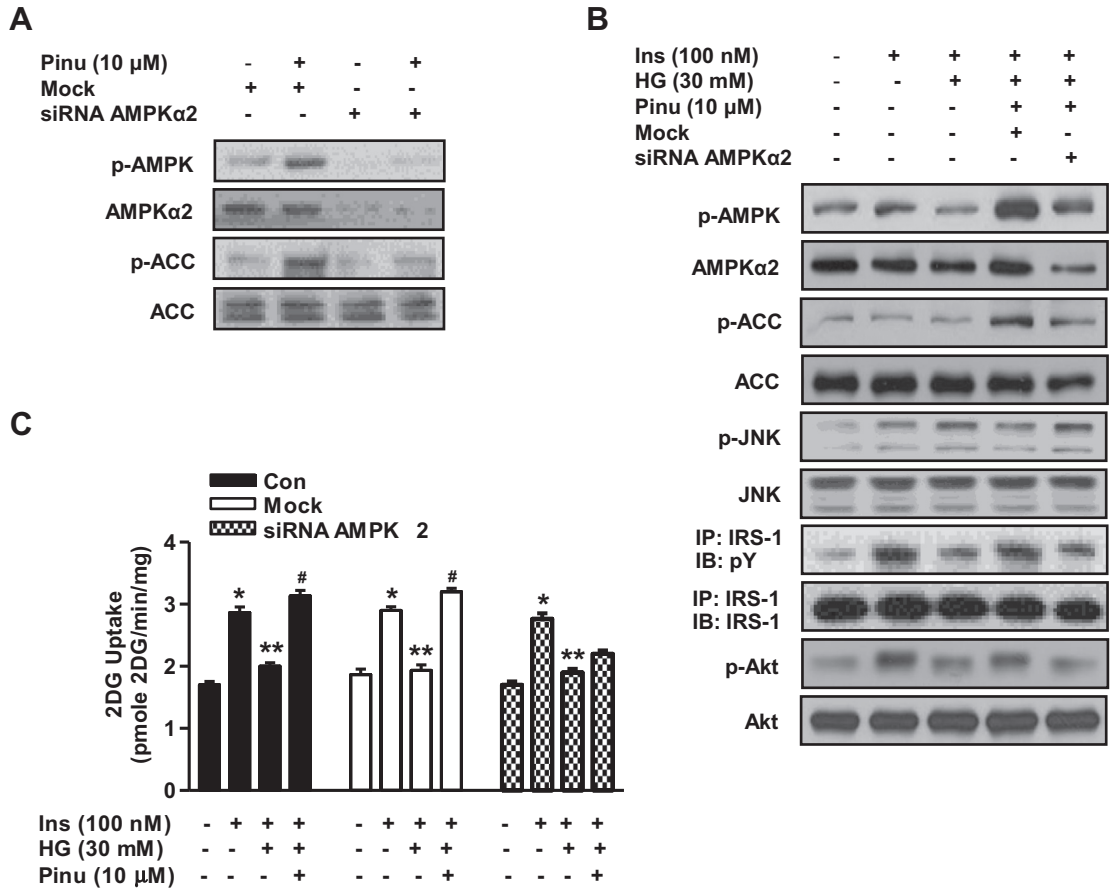

Fig. 4. Pinusolide improves high glucose-reduced insulin sensitivity via AMPK activation. (A) L6 myotubes were transfected with control siRNA (mock) or AMPKα2 siRNA for 48 h, treated with pinusolide for 2 h, and lysates were immunoblotted with the indicated phospho-specific antibodies or total antibodies as loading controls. (B) L6 myotubes were transfected with control siRNA (mock) or AMPKα2 siRNA for 48 h, incubated in serum free medium containing either 5.5 or 30 mM (high concentration) of D-glucose for an additional 24 h, pretreated with or without pinusolide for 2 h, and then stimulated with or without insulin (100 nM) for 10 min. IRS-1 tyrosine phosphorylation (pY), Akt phosphorylation, JNK phosphorylation, AMPK phosphorylation, and ACC phosphorylation and their total protein levels were determined either by immunoprecipitation following by immunoblotting or by direct immunoblotting. (C) Cells were transfected with control siRNA (mock) or AMPKα2 siRNA for 48 h, incubated in serum free medium containing either 5.5 mM (normal) or 30 mM (high concentration) of D-glucose for 24 h, pretreated with or without pinusolide for 2 h, and then stimulated with or without insulin (100 nM) for 10 min. Representative glucose uptake and quantifications of five independent experiments are shown. $^{*}P < 0.05$ versus untreated control (Student's t test) and $^{**}P < 0.05$ versus insulin-treated cells (Student's t test), $^{\#}P < 0.05$ versus untreated control and/or insulin-treated cells (Student's t test or ANOVA).

However, the effect of pinusolide on high glucose-exposed cells was blocked by AMPKα2, siRNA, suggesting that the ability of pinusolide to prevent high glucose-induced insulin resistance requires AMPK activation. Furthermore, pinusolide-reduced phosphorylation of JNK was prevented in L6 myotubes transfected with AMPKα2 siRNA under high glucose concentrations (Fig. 4B). Consistent with this, pinusolide preserved the high glucose concentration impairment of the action of insulin on glucose uptake, and this response was unaltered in cells transfected with AMPKα2 siRNA (Fig. 4C). Taken together, these results suggest that pinusolide prevents high glucose-induced insulin resistance via an AMPK-dependent mechanism.

4. Discussion

This study was undertaken to determine whether pinusolide improves insulin resistance through the LKB1–AMPK signaling pathways in muscle cells. We found pinusolide increased AMPK phosphorylation and glucose uptake, and that the siRNA-mediated knockdown of LKB1 blocked increases in AMPK phosphorylation and glucose uptake by pinusolide in L6 myotubes. Furthermore, pinusolide was found to improve insulin resistance in cells exposed to high glucose concentrations via AMPK activation. These findings strongly suggest that the beneficial effects of pinusolide on muscular glucose homeostasis are mediated mainly by AMPK.

In a previous study, it was suggested that the activation of AMPK enhances insulin sensitivity by increasing glucose uptake and lipid oxidation in skeletal muscle, and by inhibiting glucose and lipid synthesis in liver [30]. In the present study, we investigated the effect of pinusolide on AMPK activity and glucose uptake in L6 myotubes, and found that pinusolide increased AMPK phosphorylation and glucose uptake in L6 myotubes (Fig. 1). To determine whether pinusolide stimulation of glucose uptake is mediated by AMPK activation, we also examined the effect of compound C (an AMPK inhibitor). It was found that pretreatment with compound C blocked pinusolide-stimulated glucose uptake in L6 myotubes (Fig. 2).

It is well known that the tumor suppressor LKB1 activates AMPK by phosphorylating it at Thr-172 [25,26]. In the present study, pinusolide failed to induce glucose uptake in L6 myotubes treated with siRNA LKB1 (Fig. 2), LKB1 was found to be necessary for the activation of AMPK by pinusolide, which was confirmed by the prevention of this effect by LKB1 knockdown.

High glucose directly induces insulin resistance in cultured human hepatoma HepG2 cells [31], but this insulin resistance can be rescued via adenoviral-mediated expression of a Myc-tagged constitutively active mutant of AMPK [31]. Current evidence suggests

that JNK activation is closely linked to the development and pathogenesis of liver insulin resistance [32]. In addition to mediating insulin resistance via the activation of JNK, hyperglycemia exerts negative effects on insulin signaling via the serine phosphorylation of IRS-1 in animal models of type 2 diabetes [32]. In the present study, pinusolide significantly decreased high glucose-induced JNK phosphorylation, and ameliorated high glucose-reduced insulin-stimulated tyrosine phosphorylation of IRS-1 and glucose uptake in muscle cells (Fig. 3). Furthermore, AMPKα2 siRNA enhanced JNK phosphorylation, and this was reduced by pinusolide treatment under high glucose conditions (Fig. 4B). Furthermore, we found that AMPKα2 siRNA, but not control siRNA, significantly reduced the effect of pinusolide on glucose uptake under high glucose conditions (Fig. 4C), which suggests that pinusolide may increase insulin sensitivity by alleviating the inhibitory effect of JNK under high glucose conditions.

In conclusion, our results provide evidence that pinusolide benefits glucose metabolism in muscle cells, and show that AMPK pathway activation is required for pinusolide-mediated glucose uptake. In addition, we evaluated the effects of pinusolide on the insulin resistance pathway in L6 myotubes in the presence of high glucose concentration-induced insulin resistance. Furthermore, we found that pinusolide might alleviate insulin resistance by improving insulin signaling via AMPK activation and glucose uptake enhancement in insulin-resistant cells. Taken together, our findings support the notion that pinusolide might be a useful therapeutic for the treatment of metabolic disorders, such as, type 2 diabetes and their associated complications.

Acknowledgment

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References

[1] L. Rossetti, D. Smith, G.I. Shulman, D. Papachristou, R.A. DeFronzo, Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats, J Clin Invest 79 (1987) 1510–1515.
[2] H.K. Müller, M. Kellerer, B. Ermel, A. Muhlhofer, B. Obermaier-Kusser, B. Vogt, H.U. Härting, Prevention by protein kinase C inhibitors of glucose-induced insulin-receptor tyrosine kinase resistance in rat fat cells, Diabetes 40 (1991) 1440–1448.
[3] H. Maegawa, Y. Shigeta, K. Egawa, M. Kobayashi, Impaired autophosphorylation of insulin receptors from abdominal skeletal muscles in nonobese subjects with NIDDM, Diabetes 40 (1991) 815–819.
[4] D.G. Hardie, J.W. Scott, D.A. Pan, E.R. Hudson, Management of cellular energy by the AMP-activated protein kinase system, FEBS Lett 546 (2003) 113–120.
[5] D. Carling, The AMP-activated protein kinase cascade–a unifying system for energy control, Trends Biochem Sci 29 (2004) 18–24.
[6] E.J. Kurth-Kraczek, M.F. Hirshman, L.J. Goodyear, W.W. Winder, 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle, Diabetes 48 (1999) 1667–1671.
[7] R.R. Russell 3rd, R. Bergeron, G.I. Shulman, L.H. Young, Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR, Am J Physiol 277 (1999) H643–H649.
[8] G.F. Merrill, E.J. Kurth, D.G. Hardie, W.W. Winder, AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle, Am J Physiol 273 (1997) E1107–E1112.
[9] J. Mu, J.T.Jr. Brozinick, M. Bucan, M.J. Birnbaum, A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle, Mol Cell 7 (2001) 1085–1094.
[10] E.S. Buhl, N. Jessen, R. Pold, T. Ledet, A. Flyvbjerg, S.B. Pedersen, O. Pedersen, O. Schmitz, S. Lund, Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome, Diabetes 51 (2002) 2199–2206.
[11] D. Fedele, A. Tiengo, R. Nosadini, E. Marchiori, G. Briani, M.C. Garotti, M. Muggeo, Hypolipidemic effects of metformin in hyperprebetalipoproteinemia, Diabetes Metab 2 (1976) 127–133.
[12] L.S. Hermann, Metformin: a review of its pharmacological properties and therapeutic use, Diabetes Metab 5 (1979) 233–245.
[13] M.B. Davidson, A.L. Peters, An overview of metformin in the treatment of type 2 diabetes mellitus, Am J Med 102 (1997) 99–110.
[14] H.O. Yang, Y.H. Kang, D.Y. Suh, Y.C. Kim, B.H. Han, Biological and pharmacological effects of pinusolide, a novel platelet activating factor antagonist, Planta Med 61 (1995) 519–522.
[15] J.X. Zhu, Y. Wang, L.D. Kong, C. Yang, X. Zhang, Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver, J Ethnopharmacol 93 (2004) 133–140.
[16] K.A. Koo, S.H. Sung, Y.C. Kim, A new neuroprotective pinusolide derivative from the leaves of *Biota orientalis*, Chem Pharm Bull 50 (2002) 834–836.
[17] K.A. Kim, T.C. Moon, S.W. Lee, K.C. Chung, B.H. Han, H.W. Chang, Pinusolide from the leaves of *Biota orientalis* as potent platelet activating factor antagonist, Planta Med 65 (1999) 39–42.
[18] S.L. Hwang, X. Li, J.Y. Lee, H.W. Chang, Improved insulin sensitivity by rapamycin is associated with reduction of mTOR and S6K1 activities in L6 myotubes, Biochem Biophys Res Commun 418 (2012) 402–407.
[19] S.L. Hwang, H.W. Chang, I.K. Lee, B.K. Yang, J. Magae, Y.C. Chang, Ascofuranone prevents ER stress-induced insulin resistance via activation of AMP-activated protein kinase in L6 myotube cells, Biochem Biophys Res Commun 396 (2010) 967–972.
[20] B. Xue, B.B. Kahn, AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues, J Physiol 574 (2006) 73–83.
[21] B.B. Kahn, T. Alquier, D. Carling, D.G. Hardie, AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism, Cell Metab 1 (2005) 15–25.
[22] Y.C. Long, J.R. Zierath, AMP-activated protein kinase signaling in metabolic regulation, J Clin Invest 116 (2006) 1776–1783.
[23] H.J. Koh, D.E. Arnolds, N. Fujii, T.T. Tran, M.J. Rogers, N. Jessen, Y. Li, C.W. Liew, R.C. Ho, M.F. Hirshman, R.N. Kulkarni, C.R. Kahn, L.J. Goodyear, Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3, Mol Cell Biol 26 (2006) 8217–8227.
[24] K. Sakamoto, A. McCarthy, D. Smith, K.A. Green, D. Grahame Hardie, A. Ashworth, D.R. Alessi, Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction, Embo J 24 (2005) 1810–1820.
[25] S.A. Hawley, J. Boudeau, J.L. Reid, K.J. Mustard, L. Udd, T.P. Makela, D.R. Alessi, D.G. Hardie, Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade, J Biol 2 (2003) 28.
[26] A. Woods, S.R. Johnstone, K. Dickerson, F.C. Leiper, L.G. Fryer, D. Neumann, U. Schlattner, T. Wallimann, M. Carlson, D. Carling, LKB1 is the upstream kinase in the AMP-activated protein kinase cascade, Curr Biol 13 (2003) 2004–2008.
[27] J. Hirosumi, G. Tuncman, L. Chang, C.Z. Görgün, K.T. Uysal, K. Maeda, M. Karin, G.S. Hatamisligil, A central role for JNK in obesity and insulin resistance, Nature 420 (2002) 333–336.
[28] T. Purves, A. Middlemas, S. Agthong, E.B. Jude, A.J. Boulton, P. Fernyhough, D.R. Tomlinson, A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy, FASEB J 15 (2001) 2508–2514.
[29] V. Aguirre, T. Uchiuda, L. Yenush, M.F. White, The c-Jun NH_2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser^{307}, J Biol Chem 275 (2000) 9047–9054.
[30] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, J Clin Invest 108 (2001) 1167–1174.
[31] M. Zang, A. Zuccollo, X. Hou, D. Nagata, K. Walsh, H. Herscovitz, P. Brecher, N.B. Ruderman, R.A. Cohen, AMP-activated protein kinase is required for the lipid-lowering effect of metformin in insulin-resistant human HepG2 cells, J Biol Chem 279 (2004) 47898–47905.
[32] Y. Nakatani, H. Kaneto, D. Kawamori, M. Hatazaki, T. Miyatsuka, T.A. Matsuoka, Y. Kajimoto, M. Matsuhisa, Y. Yamasaki, M. Hori, Modulation of the JNK pathway in liver affects insulin resistance status, J Biol Chem 279 (2004) 45803–45809.