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Effects of chromium picolinate on glucose uptake in insulin-resistant 3T3-L1 adipocytes involve activation of p38 MAPK☆

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Abstract

Chromium picolinate (CrPic) has been discovered as a supplemental or alternative medication for type 2 diabetes, but its mechanism of action is not well understood. The purpose of this study was to explore the possible anti-diabetic mechanisms of CrPic in insulin-resistant 3T3-L1 adipocytes; the insulin resistance was induced by treatment with high glucose and insulin for 24 h. The effects of CrPic on glucose metabolism and the glucose uptake-inducing activity of CrPic were investigated. Meanwhile, the effects of CrPic on glucose transporter 4 (GLUT4) translocation were visualized by immonofluorescence microscopy. In addition, its effects on insulin signaling pathways and mitogen-activated protein kinase (MAPK) signaling cascades were assessed by immunoblotting analysis and real-time PCR. The results showed that CrPic induced glucose metabolism and uptake, as well as GLUT4 translocation to plasma membrane (PM) in both control and insulin-resistant 3T3-L1 adipocytes without any changes in insulin receptor β (IR-β), protein kinase B (AKt), c-Cbl, extracellular signal-regulated kinase (ERK), c-Jun phosphorylation and c-Cbl-associated protein (CAP) mRNA levels. Interestingly, CrPic was able to increase the basal and insulin-stimulated levels of p38 MAPK activation in the control and insulin-resistant cells. Pretreatment with the specific p38 MAPK inhibitor SB203580 partially inhibited the CrPic-induced glucose transport, but CrPic-activated translocation of GLUT4 was not inhibited by SB203580. This study provides an experimental evidence of the effects of CrPic on glucose uptake through the activation of p38 MAPK and it is independent of the effect on GLUT4 translocation. The findings also suggest exciting new insights into the role of p38 MAPK in glucose uptake and GLUT4 translocation.

Keywords: 3T3-L1 adipocytes; Chromium picolinate; Glucose transport; Insulin resistance; p38 MAPK

1. Introduction

Diabetes and its complications are major health problems throughout the world. Diabetes mellitus encompasses several types; the major types are type 1 (absolute lack of insulin) and type 2 (relative lack of insulin). Type 2 diabetes is associated with insulin resistance and relative insulin deficiency. Most of the patients with this type of diabetes may have some degree of pathophysiological insulin resistance [1,2]. Insulin resistance is characterized by a reduced ability of insulin in mediating glucose homeostasis in major insulin target tissues such as skeletal muscle, adipose tissue and liver, leading to hyperglycemia, hyperinsulinemia, dyslipidemia, abdominal obesity, fatty liver and other abnormalities [3–5]. Insulin resistance results from a combination of genetic and environmental factors and contributes to the pathogenesis of type 2 diabetes mellitus. Insulin resistance also plays a key role in other metabolic abnormalities, such as dyslipidemia and hypertension [6]. Some strategies to alleviate insulin resistance by pharmacological and nutritional supplements appear to improve insulin sensitivity in many individuals and improve glycemic control in some diabetics [7]. Chromium (Cr), a trace element which has been reported to improve abnormal carbohydrate and lipid metabolism, was proposed as a therapeutic agent for treating insulin resistance and diabetes [8–10].

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The relationship between Cr and glucose metabolism was first reported in the 1950s and suggested that brewer's yeast contained a glucose tolerance factor in which Cr is an important element that prevented diabetes in experimental animals [11]. Thereafter, various studies reported that patients deficient in Cr after long-term parenteral nutrition displayed symptoms or complications of type 2 diabetes (e.g., peripheral neuropathy, impaired glucose tolerance and elevated plasma free fatty acids) [12]. Cr was shown to play a role in the control of type 2 diabetes, steroid-induced diabetes and glucose intolerance [13–15]. Improved bioavailability of low-molecular-weight (LMW)-organic Cr complexes and the identification of the biologically active form of Cr as a complex with an oligopeptide promoted the design and evaluation of LMW-organic Cr complexes as therapeutic drugs for treating type 2 diabetes [16,17]. Cr in the soil exists in several oxidation states, principally as trivalent form, Cr(III), and hexavalent form, Cr(VI). Cr(VI) is linked with toxicity and carcinogenicity [18]. Cr(III), which is detected in most food and nutrients, is a stable essential nutrient with a very low toxicity [19]. Cr supplements are available as trivalent Cr in the chloride ($CrCl_3$), picolinate (CrPic) and nicotinate salt forms. The Cr complex of picolinic acid (CrPic), which is the effective form of Cr and the most often used dietary supplement, has been shown to increase the number of insulin receptors (IRs) and IR tyrosine-kinase activity engaged by insulin, to lower the levels of protein-tyrosine phosphatase 1B and to activate glucose transporter 4 (GLUT4) trafficking via a cholesterol-dependent mechanism [20–23]. However, the molecular mechanisms by which CrPic produces these biological effects remained enigmatic. Hence, understanding of these mechanisms is necessary for more effective use of CrPic in the treatment of type 2 diabetes.

Obesity, especially visceral adiposity, is negatively correlated with insulin sensitivity. The objectives of this study were to investigate whether CrPic could directly act on insulin-resistant 3T3-L1 adipocytes and to explore the underlying molecular mechanism of action of the signaling pathways contributing to the effects on stimulation of glucose uptake.

2. Materials and methods

2.1. Reagents and cell lines

Mouse 3T3-L1 preadipocyte cells were kindly provided by Dr Tang QQ (Key Laboratory of Molecular Medicine, Ministry of Education, Fudan University, Shanghai Medical College, China). Dulbecco's Modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO BRL (Gaithersburg, MD, USA). Newborn calf serum was purchased from Kibbutz Beit Haemek (Israel). Antibodies against IR-β, protein kinase B (AKt), extracellular signal-regulated kinase (ERK), phosphorylated ERK and GLUT4 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against phosphorylated IR-β (1158/1162/1163) was purchased from Biomol (USA). Antibodies against phosphorylated AKt (S473), phosphorylated AKt (T308), c-Cbl, phosphorylated c-Cbl (T774), p38, phosphorylated p38, c-Jun and phosphorylated c-Jun (S63) were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-GAPDH and horseradish peroxidase-conjugated secondary antibodies were purchased from Kangchen (Shanghai, China). [^{3}H]2-Deoxyglucose was purchased from Atom High-Tech (Beijing, China). The glucose assay kit was purchased from Yihua (Shanghai, China). CrPic was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). FITC-conjugated goat anti-rabbit secondary antibody, insulin, SB203580 and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture and treatment

The 3T3-L1 preadipocytes were cultured and propagated in DMEM containing glucose (25 mM) and 10% (v/v) newborn calf serum at 37°C in a 5% CO_2 atmosphere. To induce differentiation, 2-day post-confluent 3T3-L1 preadipocytes (designated Day 0) were fed with DMEM containing 10% FBS, 1 μg/ml insulin, 1 μM dexamethasone and 0.5 mM 3-isobutyl-1-methylxanthine until Day 2. Cells were then fed with DMEM supplemented with 10% FBS and 1 μg/ml insulin for 2 days, after which they were fed every other day with DMEM containing 10% FBS. With this protocol, >80% adipocyte differentiation was achieved.

Prior to induction of insulin resistance, Day 8 adipocytes were incubated in low-glucose DMEM (5.5 mM glucose) containing 10% FBS for 36–48 h. Then the cells were incubated for a period of 24 h in low-glucose DMEM (5.5 mM glucose) media containing 1% FBS and 1% (w/v) BSA without insulin as control cells or in high-glucose DMEM (25 mM glucose) media containing 1% FBS, 1% (w/v) BSA and 100 nM insulin as insulin-resistant cells. At the same time, CrPic was added in the media for 24 h. CrPic was dissolved in DMSO which was added to the control samples.

2.3. Measurement of glucose concentration

The cell culture supernatants were collected and the levels of glucose concentration were measured using the glucose assay kit for a quantitative determination.

2.4. 2-Deoxyglucose uptake

Uptake of 2-deoxyglucose (2-DOG) by the 3T3-L1 adipocytes was measured as previously described [24]. Cells were washed with KRP buffer (128 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, 5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 10 mM HEPES, pH 7.4) containing 0.1% (w/v) BSA and 5 mM glucose every 40 min for a total of 120 min at 37°C. Then cells were stimulated with 100 nM insulin in KRP buffer without glucose for 15 min or left untreated. 2-DOG (final concentration: 0.5 μCi/ml at 0.5 mM) was added and cells were incubated for 10 min. The cells were

washed three times in ice-cold phosphate buffered saline (PBS) containing 10 mM glucose; then they were lysed with 0.1N NaOH and the ^3H radioactivity was measured in a liquid scintillation counter. Each treatment condition was measured in triplicate. Glucose transport data were not corrected for nonspecific uptake. Separate experiments demonstrated that over 95% of the 2-DOG uptake in the presence of 100 nM insulin was inhibited by cytochalasin B (data not shown).

2.5. Cell lysis and immunoblotting

The cells were washed with KRP buffer three times, and then they were incubated in the presence or absence of 100 nM insulin for 15 min. Then, the cells were washed with ice-cold PBS and lysed in 1× SDS lysis buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 100 μg/ml phenylmethanesulfonyl fluoride, 10 μg/ml leupeptin and 5 mM Na_3VO_4). Protein concentration was determined by the Lowry method. Cell lysates were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked in 5% nonfat milk. After incubation of primary antibodies to IR-β, p-IR-β (1158/1162/1163), AKt, p-AKt (Ser473), p-AKt (Thr308), c-Cbl, p-c-Cbl (Tyr774), ERK, p-ERK, c-Jun, p-c-Jun (Ser63), p38, p-p38 and secondary antibodies, the membranes were developed using enhanced chemiluminescence (Amersham). The bands were scanned and quantified by TotalLab 2.01 (Nonlinear Dynamics, USA) and normalized by GAPDH.

2.6. RNA Preparations and quantitative real-time RT-PCR

Total RNA was isolated using the Trizol system (Watson, Shanghai) according to the manufacturer's guidelines. Oligo $(dT)_{15}$ primer and MMLV were used for first-strand synthesis. Real-time RT-PCR was performed with SYBR Green using Rotor-Gene 3000 (Corbett Research). PCR primers for c-Cbl-associated protein (CAP) were 5′-GGCGACATCCCTCTTCAGG-3′ and 5′-TGCTCGCGCATAAGTCGTC-3′, and β-actin was used as the internal control, whose primers were 5′-GTGAAGGTCGGAGTCAACG-3′ and 5′-TGAAGACGCCAGTGGACTC-3′. A mixture containing cDNA template (4 ng) and primers (400 nM each) underwent the following program: one cycle of 95°C for 10 min and 35 cycles of 95°C for 15 s, 62°C for 15 s and 72°C for 15 s. Expression levels of each transcript were standardized by comparing with the amount of β-actin mRNA. Quantifications were performed in duplicate and the experiments were repeated three times independently.

2.7. Immonofluorescence microscopy

The 3T3-L1 adipocytes were grown and differentiated on glass cover slips. Cells were washed with KRP buffer three times; then the cells were incubated in the presence or absence of 100 nM insulin for 15 min. After that, the cells were washed with ice-cold PBS. Then they were fixed for 15 min in 4% paraformaldehyde and washed using ice-cold PBS. They were blocked in 10% normal goat serum at 37°C for 30 min, incubated at 4°C overnight with rabbit anti-GLUT4 antibody. After three washes of 10 min each in PBS, the FITC-conjugated goat anti-rabbit secondary antibody was applied to the samples at 37°C for 1 h. The cells were visualized using an electron microscope (OLYMPUS).

2.8. Statistical analysis

The data were expressed as means±S.D. The statistical significance of differences between the non-CrPic group (0 nM CrPic) and CrPic group (100 nM CrPic) was determined using Student's *t*-test. Values of $P<.05$ were considered statistically significant.

3. Results

3.1. Effects of CrPic on glucose metabolism

The first set of experiments was aimed at investigating the influence of CrPic on glucose consumption in 3T3-L1 adipocytes for both the control and insulin-resistant cells. A concentration of 100 nM CrPic induced maximal glucose metabolism (data not shown). Therefore, this concentration was used for further experiments. For the control and insulin-resistant cells, we measured the concentration of glucose remaining in the medium of the cells with and without exposure to 100 nM CrPic for 24 h. As expected, the glucose concentration in the culture medium of insulin-resistant cells was much higher (about 10 times) than in the control cells (Fig. 1). Treatment with 100 nM CrPic reduced the glucose concentration in the medium significantly for both the insulin-resistant and control cells.

3.2. Effects of CrPic on 2-DOG uptake

Stimulation of cells with 100 nM insulin for 15 min increased glucose uptake by threefold in the control cells and 1.75-fold in the insulin-resistant cells (Fig. 2). The basal and insulin-stimulated levels of glucose uptake in the insulin-

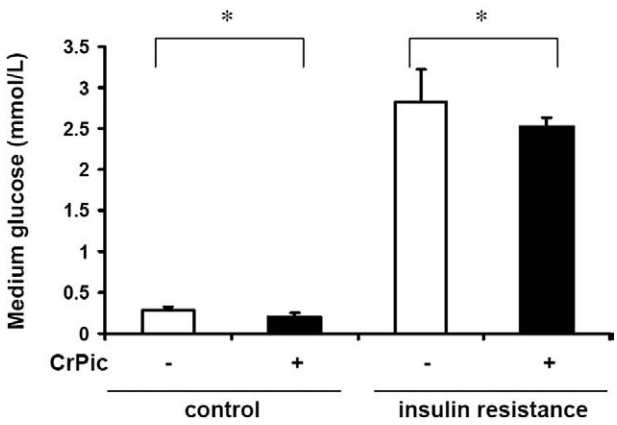

Fig. 1. Glucose metabolism in control and insulin-resistant 3T3-L1 adipocytes. Values are mean±S.D. (at least three independent experiments). Asterisks indicate a difference between CrPic-treated cells and non-CrPic-treated cells. *$P<.05$.

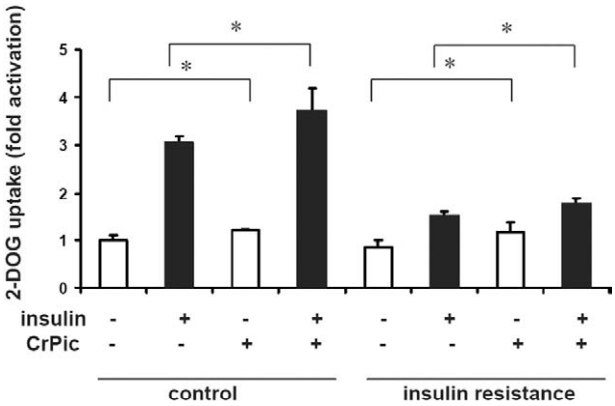

Fig. 2. Glucose (2-DOG) uptake activity in 3T3-L1 adipocytes under control and insulin-resistant conditions in the presence and absence of insulin. Mean±S.D. (triplicates) are shown. Asterisks indicate a difference between 0 nM CrPic- and 100 nM CrPic-treated cells on the basal and insulin-stimulated cells. *$P<.05$.

resistant cells were lower than in the control cells. Incubation of 3T3-L1 adipocytes with CrPic for 24 h increased the basal and insulin-stimulated glucose transport activity compared with the absence of CrPic in the control cells. In insulin-resistant cells, CrPic treatment also showed enhancement of basal and insulin-stimulated glucose uptake.

3.3. Effects of CrPic on IR signaling

To elucidate the signaling cascades that contribute to CrPic-induced glucose metabolism and glucose uptake, the effects of CrPic on intermediates of the insulin signaling pathway were investigated. After incubating with and without 100 nM CrPic for 24 h, the control and insulin-resistant 3T3-L1 adipocytes (stimulated with and without 100 nM insulin for 15 min) were probed with antibodies for total IR-β, total AKt, total c-Cbl, p-IR-β (1158/1162/1163), p-AKt (S473), p-AKt (T308) and p-c-Cbl (T774) using immunoblotting.

The expression of insulin-stimulated phosphorylation of IR-β in insulin-resistant cells was impaired as compared to the control cells (Fig. 3A), and about 14% reduction in the level of insulin-stimulated phosphorylation of IR-β (Fig. 3C) was observed. However, the control and insulin-resistant cells did not reveal any differences in the basal phosphorylation levels of IR-β. Treatment with 100 nM CrPic did not affect the basal and insulin-stimulated phosphorylation of IR-β for both the control and insulin-resistant cells. At the same time, the insulin-resistant cells, which were treated with chronic exposure to high insulin and glucose levels, showed reduction in the total IR-β (Fig. 3B), but 100 nM CrPic did not show any significant effects on the basal and insulin-stimulated expression of total IR-β.

Next, we investigated the mechanisms after IR phosphorylation. As expected, we observed that insulin dramatically stimulated AKt phosphorylation at two phosphorylation sites, Ser 473 and Thr 308 (Fig. 3D,E), in the control and insulin-resistant cells. The levels of basal and insulin-stimulated AKt phosphorylation on the sites were both impaired in the control and insulin-resistant cells. Treatment with CrPic did not increase AKt phosphorylation levels on Ser 473 and Thr 308 for either cell type. There were no significant differences in the expression of total AKt in any of the treatments (Fig. 3F).

Of the recently investigated phosphatidylinositol 3′ kinase (PI3K) pathway involved in insulin-induced glucose uptake in 3T3-L1 adipocytes, c-Cbl and CAP were shown to constitute the crucial sites [25]. Our results demonstrated that the expression of phosphorylated c-Cbl protein and CAP mRNA was significantly reduced in insulin-resistant cells as compared to control cells (Figs. 3I and 4). Insulin stimulation resulted in a significant increase in c-Cbl phosphorylation as compared to noninsulin stimulation in the control and insulin-resistant cells, without affecting the total level of c-Cbl (Fig. 3J). Nonetheless, insulin stimulation did not exhibit any effects on the gene expression of CAP. Treatment with 24-h exposure to 100 nM CrPic in the control and insulin-resistant cells had no significant effects on the expression of c-Cbl phosphorylation (Fig. 3K). CrPic treatment increased the basal and insulin-stimulated expression of CAP mRNA in the control samples; however, the increase was not statistically significant. CrPic did not affect the level of CAP mRNA in insulin-resistant cells for both basal and insulin-stimulated conditions.

3.4. Effects of CrPic on mitogen-activated protein kinase phosphorylation

CrPic enhanced glucose metabolism and glucose uptake in the control and insulin-resistant 3T3-L1 adipocytes, but it had no significant effects on the IR signaling pathway. We then investigated the effects of CrPic on p38 activity and other related mitogen-activated protein kinases (MAPKs). The results showed that CrPic did not induce the basal or insulin-stimulated activation of ERK (Fig. 5A,C) and c-Jun (Fig. 5D,F) in the control or insulin-resistant cells. It was obvious, however, that in insulin-resistant adipocytes, the basal and insulin-stimulated levels of p-p38 decreased by about 20% as compared to the control cells (Fig. 5G,I). The results also indicated that treatment with CrPic resulted in a significant increase of basal and insulin-stimulated activation of p38 in both the control and insulin-resistant cells. The expression of all the three total MAP kinases was unaffected by the treatment of CrPic in the presence or absence of insulin in the control and insulin-resistant cells (Fig. 5B,E,H).

3.5. Effects of SB203580 on CrPic-induced p38 activation and glucose uptake

SB203580, a specific inhibitor of p38 MAPK, has been shown to inhibit insulin-stimulated glucose uptake in 3T3-L1 adipocytes and L6 myotubes [26]. Ten micromolar SB203580 was added to the cells 1 h prior to the addition of CrPic and remained in the medium for the entire 24-h

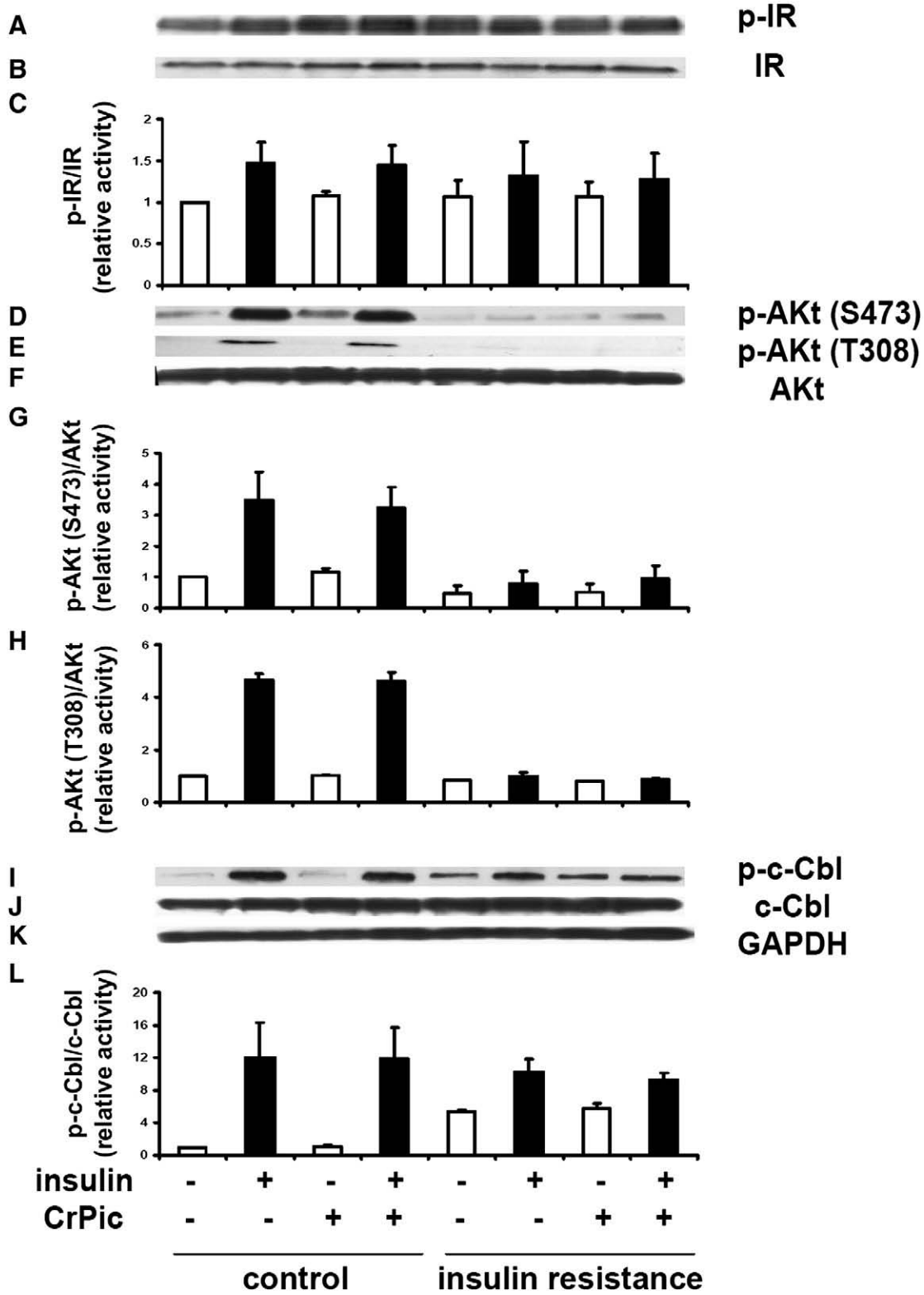

Fig. 3. Insulin and CrPic (in)dependent IR-β phosphorylation (A–C), AKt phosphorylation (D–H) and c-Cbl phosphorylation (I,J,L) in control and insulin-resistant 3T3-L1 adipocytes. The error bars are obtained from three independent experiments. They were normalized by GAPDH.

incubation period with CrPic. Under this condition, the results showed that SB203580 inhibited CrPic-induced p38 phosphorylation in the control and insulin-resistant cells (Fig. 6C). Meanwhile, the glucose transport activity induced by CrPic was also inhibited by the treatment of SB203580 (Fig. 6D).

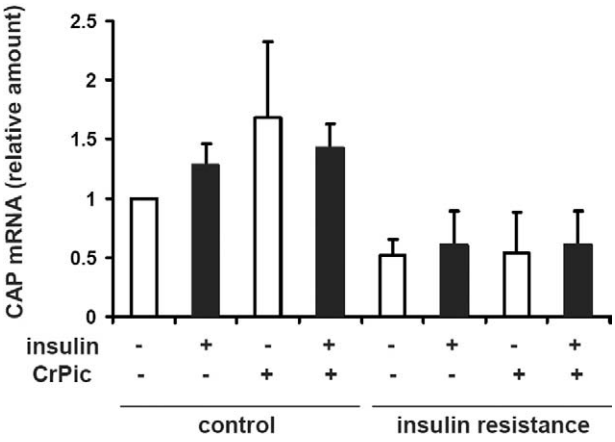

Fig. 4. Expression of CAP in the control and insulin-resistant 3T3-L1 adipocytes. These data are obtained from three independent experiments.

3.6. Effects of SB203580 on CrPic-induced GLUT4 translocation

We observed the plasma membrane (PM) localization of GLUT4 by using the immunofluorescence microscopy technique to determine whether the inhibition in CrPic-induced glucose uptake observed in the presence of the SB203580 was due to lower GLUT4 translocation. Consistent with the results observed from glucose metabolism and uptake, CrPic and insulin increased the translocation of GLUT4 from intracellular pools to the PM in both the control and insulin-resistant 3T3-L1 adipocytes. However, the effect of the CrPic-stimulated GLUT4 translocation was less significant than that of insulin (Fig. 7A–H). In insulin-resistant cells, the translocation of GLUT4 was less obvious than that of the control cells. After the treatment with SB203580, the stimulation of GLUT4 translocation by CrPic and insulin in the control and insulin-resistant cells was unaffected (Fig. 7I–P).

4. Discussion

Cr, an essential trace element, has been shown to have therapeutic effects for treating type 2 diabetes [27]. The ability of Cr in enhancing insulin-stimulated glucose uptake in normal 3T3-L1 adipocyte and C2C12 myoblast cell lines has been reported previously [23,28,29]. However, the exact mode of action of Cr is still controversial and remains unclear. This study was undertaken to explore the potential mechanism of action of Cr in treating type 2 diabetes in insulin-resistant 3T3-L1 adipocytes which mimic the pathophysiological abnormality of type 2 diabetes. Understanding the molecular basis of the mechanism of action of Cr is of crucial importance because this can help us to determine whether Cr supplementation can be used as an effective adjunct therapy for type 2 diabetes.

We examined the effects of CrPic on insulin-resistant 3T3-L1 adipocytes since it has been previously reported that treating hyperglycemia with insulin could induce the development of insulin resistance in 3T3-L1 adipocytes [30]. Thus, adipocytes, which are among the major target cells of insulin, were used as a model system for studying insulin resistance *in vitro*. Our studies showed that CrPic enhanced basal and insulin-stimulated glucose uptake in both the control and insulin-resistant cells. In addition, we also observed that CrPic accelerated glucose utilization, as reflected by the rapid loss of glucose from the incubation medium. These data demonstrated that treatment with CrPic could partially reduce hyperglycemia and insulin-induced insulin resistance. These effects of CrPic were independent of insulin, indicating that CrPic could be regarded as an insulin analog.

In pursuing the underlying mechanism by which CrPic exerted its effects on glucose uptake and metabolism, we examined whether CrPic could reverse the high glucose and insulin-induced impairments in the insulin signaling cascades. It is well known that the insulin-activated signal transduction mechanism that stimulates glucose uptake is initiated by the binding of insulin to IR, followed by receptor tyrosine autophosphorylation [31]. The activated IR tyrosine kinase phosphorylates IR substrate-1 on tyrosine residues which in turn binds to PI3K, thereby recruiting the 3′ phosphoinositide-dependent kinase-1 (PDK-1). PDK-1 phosphorylates and stimulates both PKB/AKt and the atypical protein kinase C (PKC) λ/ζ [32]. Then the PKB phosphorylation on Thr 308 and Ser 473 as well as the atypical PKC λ/ζ promotes the translocation of GLUT4. An additional PI3K independent of insulin signaling required for GLUT4 translocation and glucose uptake has been identified. Phosphorylation of the adaptor protein c-Cbl by IR and the involvement of CAP play crucial roles in this pathway [25].

We showed that chronic hyperglycemia and hyperinsulinemia could result in the loss of IR expression and the phosphorylation level of IR-β in the presence and absence of insulin stimulation. However, CrPic did not enhance the basal and insulin-stimulated phosphorylation of IR-β.

PI3K activity is pivotal for insulin-stimulated GLUT4 translocation and glucose uptake. Nevertheless, CrPic did not change the phosphorylation of AKt on Ser 473 and Thr 308. So these investigations suggest that CrPic may act on different targets residing in other pathways.

In addition, CrPic treatment had no effect on the expression of c-Cbl phosphorylation and CAP mRNA in basal and insulin-stimulated conditions. These results demonstrate that the enhancement of glucose transport and metabolism by CrPic does not involve the traditional insulin signaling pathways. These results are consistent with the investigation on normal 3T3-L1 adipocytes, which reported that CrPic had no effects on the traditional insulin signaling pathway [23].

Furthermore, our study suggests that ERK and c-Jun activation were not impaired in insulin resistance induced by hyperglycaemia and hyperinsulinemia in 3T3-L1 adipocytes.

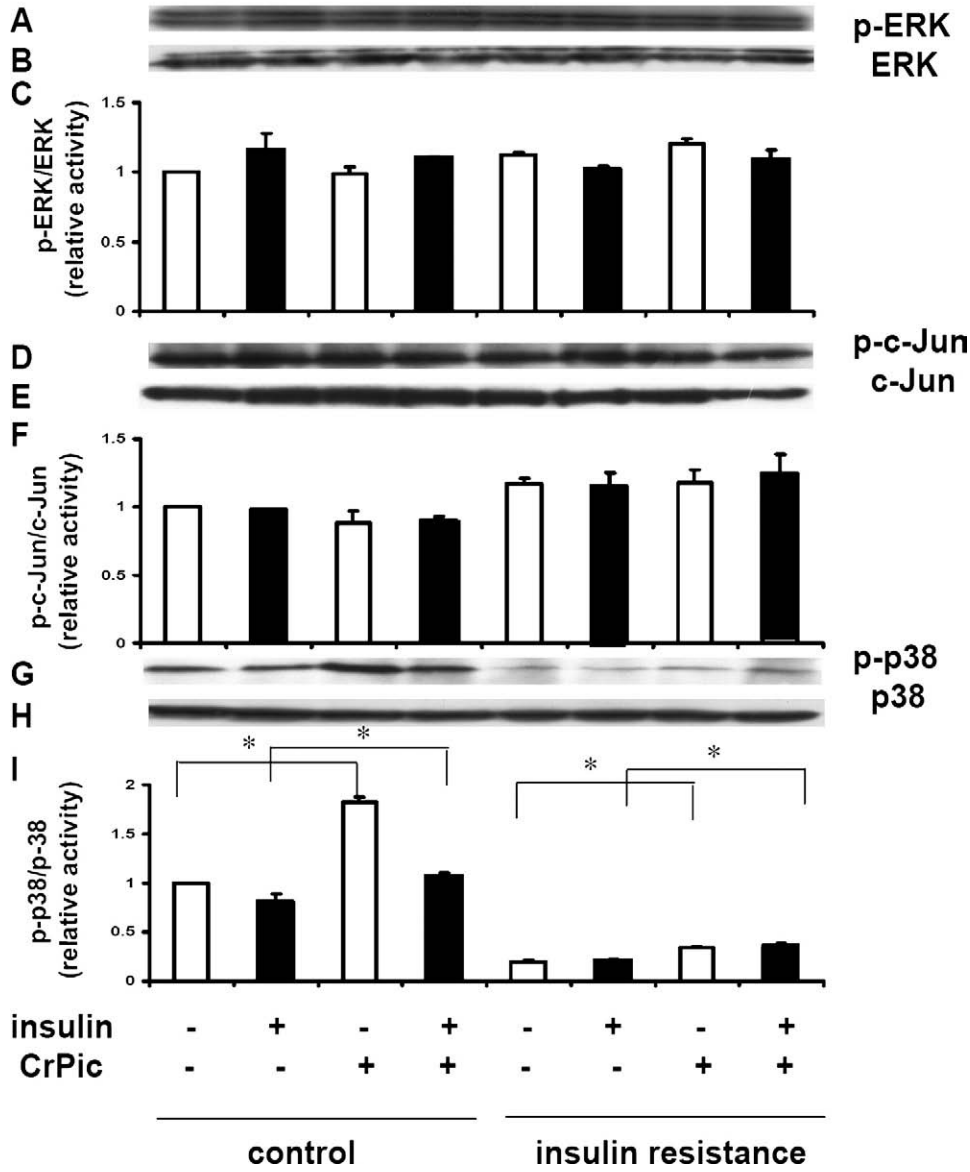

Fig. 5. Activation of p38 MAPK (G–I) and the effects on ERK phosphorylation (A–C) and c-Jun phosphorylation (D–F) of CrPic in control and insulin-resistant 3T3-L1 adipocytes. The error bars are representative for three independent experiments. The asterisk indicates a difference between CrPic-treated cells and non-CrPic-treated cells. *$P<.05$.

These findings are consistent with other findings [33]. The basal and insulin-stimulated levels of ERK and c-Jun were unaffected by treatment with CrPic. Recent studies have proposed a potential role of p38 MAPK activation in glucose uptake induced by insulin [34,35]. Moreover, some investigations have suggested that after GLUT4 translocation towards the PM, there was a secondary p38 MAPK-dependent step leading to an enhancement of insulin-induced glucose uptake [36]. The specific p38 MAPK inhibitor, SB203580, affects glucose turnover by the insulin-responsive GLUT4 transporter in 3T3-L1 adipocytes [37]. So p38 MAPK may be a crucial target for many antidiabetic drugs. Some drugs, such as metformin and arsenite, have been tested to exert antidiabetic effects through the activation of p38 MAPK [38,39]. Activation of p38 MAPK as reflected by the level of p38 MAPK phosphorylation was impaired in insulin-resistant cells as compared to control cells. Interestingly, treatment of control and insulin-resistant cells with CrPic enhanced the basal and insulin-stimulated p38 MAPK activation. These results are consistent with the results obtained on glucose uptake. Pretreatment of cells with a concentration of 10 μM SB203580 blocked CrPic-induced enhancement of p38 phosphorylation as well as glucose uptake levels in the control and insulin-resistant cells. Based on these data, we conclude that the p38 MAPK activation may be involved in CrPic-induced glucose transport. CrPic-induced glucose uptake is sensitive to the treatment of SB203580, and the activation of p38 MAPK is impaired in

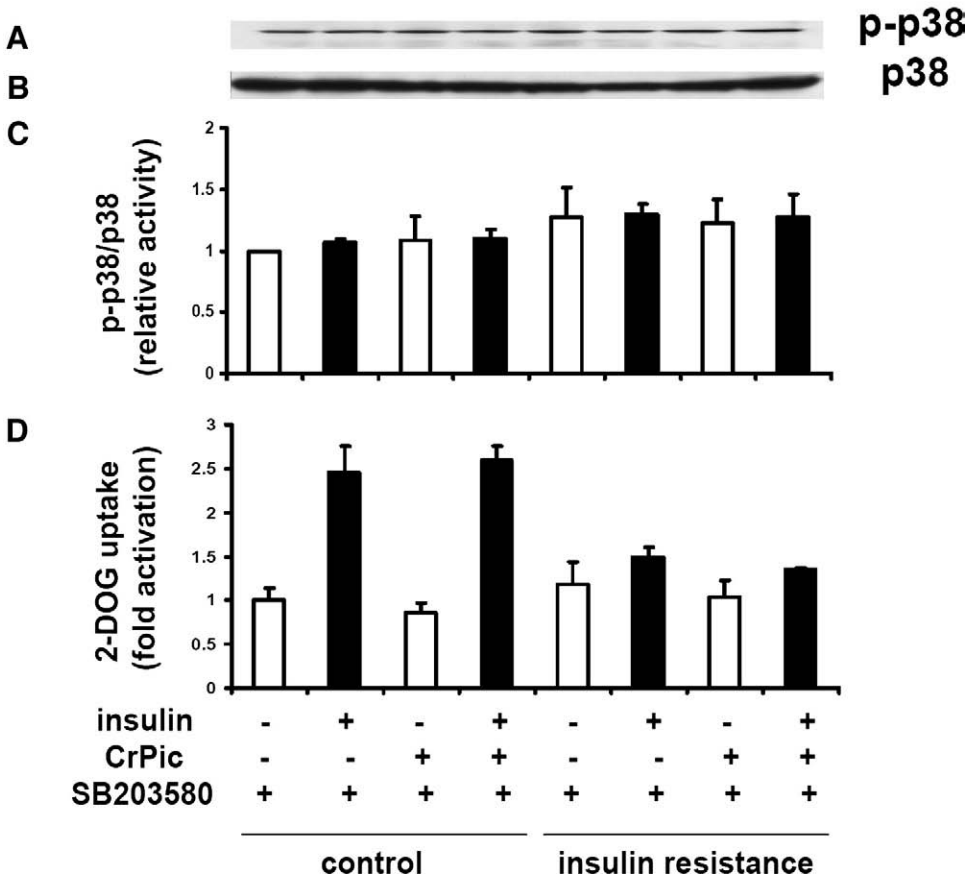

Fig. 6. Activation of p38 (A–C) and 2-DOG uptake (D) in control and insulin-resistant 3T3-L1 adipocytes in the presence of SB203580. SB203580 is a specific p38 MAPK inhibitor. Data from (C) represent the densitometric analysis relative to control samples of three independent experiments. Values from (D) are mean±S.D. (at least three independent experiments).

insulin-resistant cells. These findings may provide additional data suggesting a role of p38 MAPK in glucose uptake and the development of insulin resistance in 3T3-L1 adipocytes. Cr was reported to have the ability to enhance the level of p38 MAPK phosphorylation, but the relationship between this effect and its antidiabetic effects was not investigated [40]. The mechanisms by which CrPic enhanced insulin-dependent and insulin-independent p38 MAPK phosphor-

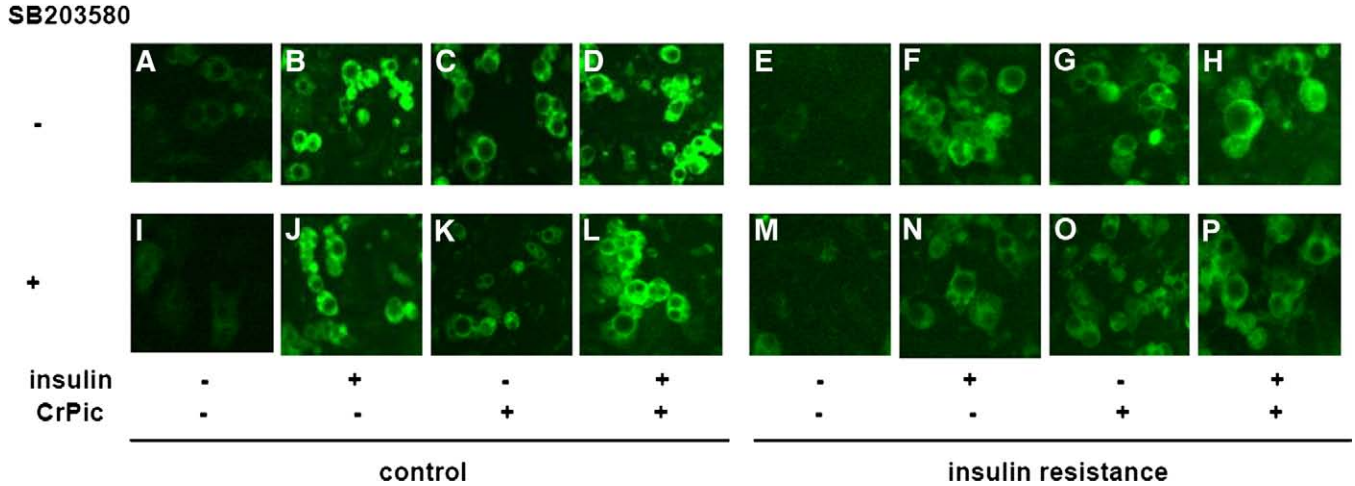

Fig. 7. Visualization of CrPic-induced GLUT4 translocation in control and insulin-resistant 3T3-L1 adipocytes in the presence of SB203580. The cellular localization of GLUT4 was observed under a fluorescence microscope. Representative fields are shown. The figure shown is one of the three independent experiments. All three experiments showed similar results.

ylation are unclear. This requires further research on the mechanism of CrPic in activating p38 MAPK.

It is widely recognized that insulin stimulates glucose uptake via the translocation of GLUT4 to the PM in muscle and adipose tissues. Nevertheless, previous observations revealed that the inhibitory effect of SB203580 on glucose uptake is not due to a nonspecific effect on insulin signaling pathways nor directly on GLUT4 translocation [26]. Other reports showed that GLUT4 is activated following its translocation, and this activation is prevented by SB203580; p38 MAPK contributes to enhancing the intrinsic activity of GLUT4 [41,42]. In our study, we demonstrate a similar effect on CrPic-induced GLUT4 translocation. In contrast to its attenuated effect on glucose uptake, SB203580 did not affect the CrPic-stimulated GLUT4 translocation into the PM, indicating that the translocation of GLUT4 is determined by other factors. Our data show a similar contribution of p38 MAPK activity in combination with GLUT4 translocation in insulin- and CrPic-induced glucose uptake at the level of modulating the GLUT4-mediated transport activity. Chen et al. [23] reported that CrPic activates GLUT4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. In our investigation, we postulate that CrPic may regulate the intrinsic activity of GLUT4 through p38 MAPK pathway.

In summary, we showed that CrPic increased the basal and insulin-stimulated glucose uptake and metabolism as well as GLUT4 translocation to PM in both the control and insulin-resistant 3T3-L1 adipocytes. CrPic increased the basal and insulin-stimulated p38 MAPK activation. SB203580 partially inhibited the CrPic-induced glucose transport without affecting the translocation of GLUT4 induced by CrPic. Based on these results, we postulate that the mechanism of action of CrPic is independent of insulin and CrPic may have a possible antidiabetic effect in insulin-resistant 3T3-L1 adipocytes through the involvement of p38 MAPK. To our knowledge, this is the first report showing that CrPic increases glucose transport in insulin-resistant 3T3-L1 adipocytes. It provides evidence for a novel molecular mechanism by which CrPic's antidiabetic effect is related to the stimulation of p38 MAPK in both the control and insulin-resistant 3T3-L1 adipocytes. Elucidation of the signaling components involved in the effects of CrPic should provide exciting new insights into the role of p38 MAPK in glucose uptake. The p38 MAPK can be a potential therapeutic target for novel antidiabetic drugs. We believe that there will be increased efforts to elucidate the exact mechanisms of CrPic action in the future.

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