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ARTICLE

cis-9,*trans*-11-Conjugated Linoleic Acid Activates AMP-Activated Protein Kinase in Attenuation of Insulin Resistance in C_2C_{12} Myotubes

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Obesity is a key risk factor in the development of insulin resistance (IR). This study is to investigate the IR attenuating effect and the molecular mechanism of *cis*-9,*trans*-11-conjugated linoleic acid (c9, t11-CLA). This study was performed with a palmitate-induced IR model using C_2C_{12} myotubes and showed that c9,t11-CLA increased insulin-stimulated and basal (non-insulin-stimulated) glucose uptake of IR myotubes. c9,t11-CLA also up-regulated the levels of phosphorglycogen synthase, phosphoracetyl CoA carboxylase, and carnitine palmitoyltransferase-1 while down-regulating the level of pyruvate dehydrogenase kinase 4 under insulin-stimulated and basal conditions. However, c9,t11-CLA did not affect protein kinase B/Akt (Akt). These results suggested that c9,t11-CLA induced an insulin-independent enhancement of glucose and fatty acid metabolism. Furthermore, there was a dose- and time-dependent increase in the expression of phosphor-AMP-activated protein kinase (AMPK), whereas LKB1, the upstream kinase of AMPK, was unchanged. Collectively, c9,t11-CLA attenuated palmitate-induced IR by increasing the consumption of glucose and fatty acid, the mechanism involving the direct activation of AMPK.

KEYWORDS: *cis*-9,*trans*-11-Conjugated linoleic acid; insulin resistance; free fatty acid; glucose and FA metabolism; AMP-activated protein kinase

INTRODUCTION

Obesity is a key risk factor in the development of both insulin resistance (IR) and type 2 diabetes. Obesity leads to hyperlipidemia with specifically increased free fatty acids (FFA) in the blood. The elevated level of FFA is responsible for the impairment of glucose utilization and a decrease of insulin sensitivity, which both contribute to the establishment of IR (*1*−*4*). Because of the importance of IR and FA abnormalities in the development of type 2 diabetes, many investigators have confirmed that dyslipidemia, hyperglycemia, and IR can aggravate each other, which means they are involved in an vicious circle (*5*−*7*). Therefore, an ideal solution for obesity-induced IR would be simultaneous reduction of blood FFA and glucose (*5*, *8*, *9*).

Recently, AMP-activated protein kinase (AMPK) has attracted a great deal of interest due to its regulatory effects on intracellular fatty acid (FA) oxidation and insulin sensitivity (*10*), and it is considered to be a master switch in the regulation of glucose and FA metabolism (*11*). Activated AMPK influences a variety of metabolic processes, leading to reduced energy storage and increased energy production (*10*) by enhancing the consumption of glucose and FA and, thus, reducing blood levels of FFA and glucose. Therefore, AMPK is considered to be a key target for attenuation of IR, and hence nutrients that can enhance its activity are of significant interest.

Conjugated linoleic acid (CLA), a group of positional and geometric isomers of the omega-6 essential FA [18:2(n-6)] naturally present in ruminant meat and dairy products, has received much attention for its biological pleiotropic effects, such as anticancer, antiatherosclerotic, and weight reduction activities (*12*−*14*). To date, CLA is shown to promote FA metabolism and decrease body fat. However, there is a contradiction concerning the effects of CLA on glucose metabolism; some studies indicate that CLA contributes to IR (*15*, *16*), whereas others suggest that CLA increases insulin sensitivity (*17*−*20*). These conflicting results may derive from different experimental designs and CLA isomeric composition, because the metabolic health effects of CLA are isomer-specific. *cis*-9,*trans*-11-Conjugated linoleic acid (c9,t11-CLA), a major isomer of CLA in foods, has been proved to increase insulin sensitivity (*21*, *22*). Earlier studies of this activity were focused mostly on its lipid-regulation (*21*) and anti-inflammatory effects (*22*). Nevertheless, the effects of c9,t11-CLA on glucose and FA metabolism have seldom been investigated concurrently and, to our knowledge, the influence of c9,t11-CLA on AMPK has not been reported.

In this study, we determined whether the anti-IR potential of c9,t11-CLA was mediated through the activation of AMPK. The results revealed that c9,t11-CLA attenuated IR by promoting both glucose and FA metabolism and demonstrated for the first

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time that the AMPK-activating effect was involved in the insulin sensitization effect of CLA.

MATERIALS AND METHODS

Materials. c9,t11-CLA (98% pure) was purchased from Matreya (Pleasant Gap, PA). The C_2C_{12} cell line was purchased from American Type Culture Collection. Antibodies against glycogen synthase (GS), phosphor-GS (p-GS), AMPKα, phosphor-AMPKα (p-AMPKα), AMPKβ, phosphor-AMPKβ (p-AMPKβ), acetyl CoA carboxylase (ACC), phosphor-ACC (p-ACC), protein kinase B/Akt (Akt), and phosphor-Akt (p-Akt) were purchased from Cell Signaling (Beverly, MA). Antibodies against p-LKB1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against pyruvate dehydrogenase kinase 4 (PDK4), LKB1, and carnitine palmitoyltransferase-1 (CPT-1) were purchased from Abcam Inc. (Cambridge, MA) and Life-Span Biosciences (Seattle, WA), respectively. Alkaline phosphatase−goat ant-irabbit IgG and deoxy-D-glucose, 2-[1,2-^{3}H(N)] ([2-^{3}H]-DG) were purchased from Promega (Madison, WI) and Perkin-Elmer Life and Analytical Sciences (Boston, MA), respectively. Palmitate, cytochalasins, insulin, MTT assay kit, and 2-deoxy-D-glucose (2-DG) were purchased from Sigma-Aldrich (St. Louis, MO). The polyvinylidene difluoride (PVDF) membrane was purchased from Millipore Corp. (Billerica, MA).

Fatty Acid Preparation. Palmitate (25 mM) was dissolved in NaOH (50 mM) at 70 °C and mixed with 10% (w/v) bovine serum albumin (BSA) at 55 °C, as 5 mM reserving liquid. Then the reserving liquid was diluted 1:20 (v/v) with serum-free Dulbecco's modified Eagle's medium (DMEM) to obtain 0.25 mM palmitate solution. The c9,t11-CLA was dissolved in dimethyl sulfoxide (DMSO) and diluted to different concentrations (0, 20, 30, and 40 μM) with serum-free DMEM containing 0.25 mM palmitate.

Cell Culture. C_2C_{12} is a mouse myoblast cell line, and it was maintained in DMEM (4.5 mg/mL glucose) with 10% (v/v) fetal calf serum in a 95% air/5% CO_2 atmosphere. When 80% confluence was reached, the culture medium was changed to DMEM with 2% (v/v) horse serum to initiate myogenic differentiation. After 3−4 days, fully mature myotubes were exposed to palmitate (0.25 mM) with c9,t11-CLA at different concentrations (0, 20, 30, and 40 μM) for 18 h. In parallel, myotubes treated with an equivalent volume of DMSO but without palmitate were used as the normal control. The concentration of the solvent DMSO in the incubation buffer was 0.1%. If the cells needed to be exposed to insulin, the insulin (100 nM) was added into medium 18 h before cells were harvested. To test the time-dependent effect of CLA, the myotubes were incubated with medium supplemented with palmitate (0.25 mM) and c9,t11-CLA (40 μM) for 2, 4, 8, 12, and 18 h, respectively. For cellular toxicity, the MTT assay was performed according to the manufacturer's instructions to ensure that the treatment with palmitate or CLA did not affect cellular viability. Cells were seeded on a 96-well plate and differentiated to myotubes. Palmitate (0.25 mM), various concentrations of CLA, and 0.25 mM palmitate with various concentrations of CLA were added into the wells and incubated for 18, 24, and 36 h for each condition. The culture medium was replaced on alternate days, and the cells were kept in a medium free of serum during treatment. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (5%, 20 μL) was added to the wells 4 h before the end of incubation. Medium and reagents were aspirated, and 98% DMSO was added. After 15 min of shaking, the optical density at 570 nm was measured using a plate reader (ELx 800 automated microplate reader; Bio-Tech Instruments). Cell viability was calculated from the optical density readings, using control cells as 100%.

Glucose Uptake Assays. Glucose transport was determined by measuring the uptake of [2-^{3}H]-DG. Cells were cultured in 24-well plates and differentiated to myotubes. After incubation for 18 h with 0.25 mM palmitate and various concentrations of c9,t11-CLA in serum-free DMEM (1.1 mg/mL glucose) containing 0.5% (w/v) BSA, cells were washed twice using transport buffer (20 mM Hepes, 137 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4). Then cells were incubated in transport buffer at 37 °C in the absence or in the presence of 100 nM insulin for 30 min before the addition of [2-^{3}H]-DG (final concentration = 1 μCi/mL) and 2-DG (final concentration = 0.1 mM). Uptake was terminated 10 min later by aspiration of the solution. Cells were washed three times with ice-cold phosphate-buffered saline, and radioactivity was determined by cell lysis in 0.05 M NaOH, followed by scintillation counting (Beckman, LS6500). Aliquots from each well were used to measure total protein concentration by the Bradford assay. Nonspecific counts, determined in the presence of 10 mM cytochalasin B, were subtracted from each value.

Western Blot. Proteins were separated by SDS-PAGE [8 or 5% (w/v) polyacrylamide], transferred to PVDF membrane, and blocked in 1% BSA and TBS-T (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 0.5 h at room temperature. Membranes were incubated overnight at 4 °C with primary antibodies in TBS-T. Membranes were washed three times in TBS-T for 10 min each time and incubated with alkaline phosphatase−goat anti-rabbit IgG for at room temperature for 1 h, followed by two washes in TBS-T and one in TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) for 10 min each time. Proteins were quantified by densitometry.

Statistics. All data were presented as mean ± standard deviation (SD). Statistical analysis used one-way ANOVA, and the level of statistical significance was set at $P \leq 0.05$. All analysis was done with SPSS software.

RESULTS

c9,t11-CLA Improved Insulin Sensitivity and Increased Glucose Uptake. To determine how c9,t11-CLA affects insulin sensitivity and glucose uptake in IR myotubes, we measured [2-^{3}H]-DG uptake in the different experimental groups. As shown in **Figure 1A**, the insulin-stimulated glucose uptake was much higher in the absence of c9,t11-CLA and palmitate than that in the basal group. After treatment with 0.25 mM palmitate, the glucose uptake was decreased significantly, indicating that establishment of the IR model was due to the administration of palmitate. The increase of glucose uptake after the administration of c9,t11-CLA was dose-dependent. Therefore, we believe that c9,t11-CLA increased insulin sensitivity in the IR model. As can be seen in **Figure 1B**, the increased uptake of glucose under basal conditions was dependent on the concentration of c9,t11-CLA. These findings suggest that c9,t11-CLA increased glucose uptake even in the absence of insulin.

c9,t11-CLA Altered the Expressions of Molecular Markers of Glucose Metabolism. Two pathways are involved in glucose metabolism in skeletal muscle: anabolism (synthesis of glycogen) and catabolism (aerobic oxidation and glycolysis). We know that GS is a rate-limiting enzyme in glycogen synthesis and that PDK4 plays an important role in glucose oxygenolysis. In this study, we determined the levels of GS, p-GS, and PDK4 and found that the ratio p-GS/GS was increased and the level of PDK4 was decreased by c9,t11-CLA treatment in a dose-dependent manner (**Figure 2**). Because p-GS is the inactive form of the enzyme, the activity of GS was down-regulated by c9,t11-CLA. Meanwhile, PDK4 is related to the phosphorylation and inactivation of pyruvate dehydrogenase complex (PDC), which is in charge of a rate-limiting step of glucose oxidation. Therefore, compared with the IR group (treated only with palmitate), the c9,t11-CLA-treated groups showed the reduction of PDK4, and it probably led to the increased activity of PDC, followed by the enhancement of glucose oxidation.

c9,t11-CLA Altered the Expressions of Molecular Markers of FA Oxidation. To investigate the effects of c9,t11-CLA on FA metabolism, we determined the levels of ACC, p-ACC, and CPT-1, which are important molecular markers of FA oxidation. As shown in **Figure 3**, the ratio p-ACC/ACC and the level of CPT-1 in the palmitate-treated groups were significantly lower than in the normal control. However, the intervention of c9,t11-CLA resulted in a dose-dependent increase of the ratio p-ACC/ACC and the level of CPT-1, which indicated that FA β-oxidation was enhanced by c9,t11-CLA.

c9,t11-CLA Influenced Glucose and FA Metabolism Independent of Insulin. We found that in the presence of insulin, 40 μM c9,t11-CLA also markedly increased the ratio p-GS/GS and the level of

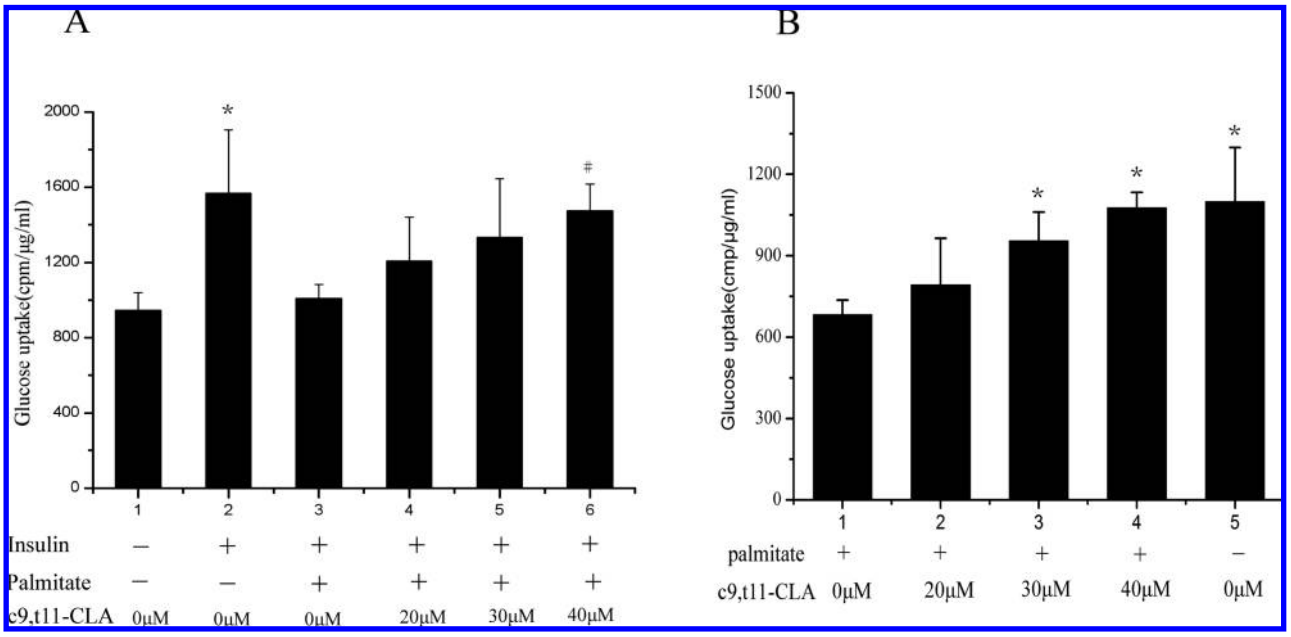

Figure 1. c9,t11-CLA promoted glucose uptake in IR C_2C_{12} myotubes. The fully differentiated myotubes were incubated in the absence or in the presence of 0.25 mM palmitate with various concentrations of c9,t11-CLA. (**A**) c9,t11-CLA enhanced the insulin sensitivity of IR myotubes. The [2-^{3}H]-DG uptake of the myotubes was determined after treatment in the absence (bar 1) or in the presence (bars 2−6) of 100 nM insulin for 30 min. ∗, $P < 0.05$ versus normal non-insulin-stimulated myotubes (bar 1) or insulin-stimulated myotubes incubated with palmitate only (bar 3); #, $P < 0.05$ versus insulin-stimulated myotubes incubated with palmitate only (bar 3). (**B**) c9,t11-CLA increased glucose uptake of IR myotubes under basal conditions. ∗, $P < 0.05$ versus myotubes incubated with palmitate only (bar 1). Data are expressed as mean ± SD of three independent experiments.

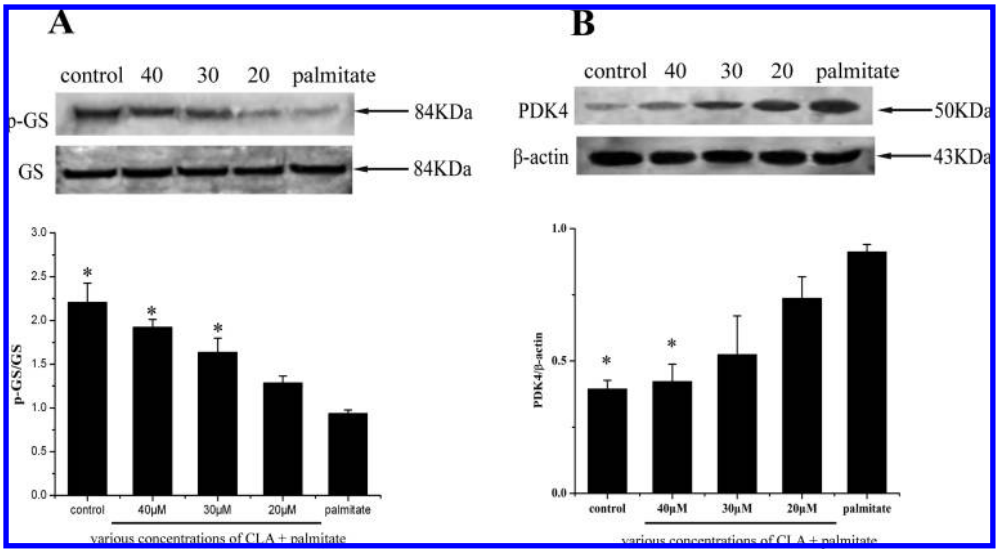

Figure 2. Effects of c9,t11-CLA on GS and PDK4 in IR myotubes. The myotubes were incubated in the absence (control) or in the presence of 0.25 mM palmitate. The groups in the presence of palmitate were treated with 40, 30, or 20 μM c9,t11-CLA or without c9,t11-CLA. (**A**) The protein levels of p-GS and GS were quantified by densitometry, and values of p-GS relative to GS are expressed in the bar chart. (**B**) The protein levels of PDK4 and β-actin were quantified by densitometry, and values of PDK4 relative to β-actin are expressed in the bar chart. ∗, $P < 0.01$ versus myotubes incubated with palmitate only. Data are expressed as mean ± SD of three independent experiments.

CPT-1 and decreased the level of PDK4. The results were similar in the groups without insulin (**Figure 4A−C**). Moreover, after the treatment of c9,t11-CLA, Akt, an important protein kinase of the insulin signaling pathway, was not significantly changed in either condition (+ or − insulin) (**Figure 4D**). These results indicate that c9,t11-CLA influenced glucose and FA metabolism in an insulin-independent manner.

c9,t11-CLA Activated AMPK by a Direct Route. AMPK, an upstream marker of ACC, is an important energy sensor in metabolism, with LKB1 being its upstream kinase. To further investigate the mechanism by which c9,t11-CLA exerted its metabolic regulatory activity, we examined the effect of the treatment with c9,t11-CLA on the level and phosphorylation status of AMPK and LKB1. As shown in **Figure 5**, c9,t11-CLA elevated the level of p-AMPKα dose-dependently and time-dependently, whereas the β-subunit of AMPK was unchanged (data not shown). In contrast, neither the content of LKB1 nor the ratio p-LKB1/LKB1 was altered significantly across the treatments (data not shown). These results indicate that c9, t11-CLA activated AMPK by phosphorylating the α-subunit directly and that LKB1 might not be the target in the course's process.

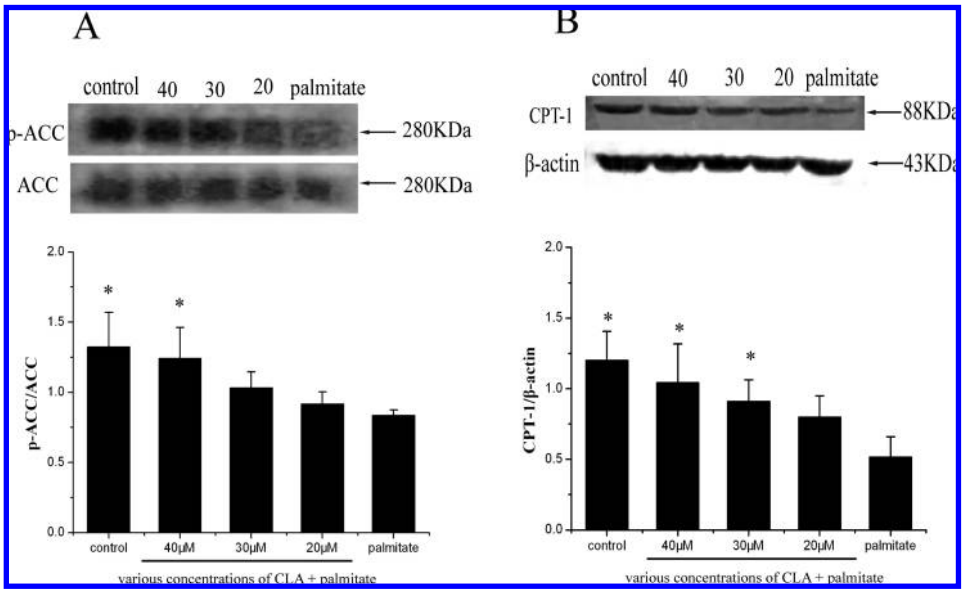

Figure 3. Effect of c9,t11-CLA on ACC and CPT-1 in IR myotubes. Treatments of each group were as described for **Figure 2**. (**A**) Levels of p-ACC and ACC were quantified by densitometry, and values of p-ACC relative to ACC are expressed in the bar chart. (**B**) Levels of CPT-1 and β-actin were quantified by densitometry, and values of CPT-1 relative to β-actin are expressed in the bar chart. $*$, $P < 0.05$ versus myotubes incubated with palmitate only. Data are expressed as mean $\pm$ SD of three independent experiments.

DISCUSSION

IR associated with obesity is reaching epidemic proportions worldwide. The results of our earlier study suggested that CLA increased insulin sensitivity (*20*). However, the metabolic regulatory effect of CLA is isomer-specific. One of the major isomers of CLA is c9,t11-CLA. Recently, it was found that c9,t11-CLA could relieve hyperlipidemia in the obesity-induced metabolic syndrome (*21*) and attenuate IR via anti-inflammatory effects in white adipose tissue (*22*). Although these actions of c9,t11-CLA were mentioned in the reports of some studies, details of the mechanism have not been fully investigated. On the basis of the present study, we propose possible explanations for the mechanisms of c9,t11-CLA-mediated IR attenuation, and the novelty of our studies was that we elucidated how c9,t11-CLA increased insulin sensitivity by influencing the energy metabolic homeostasis.

Skeletal muscle is a metabolically active tissue, and a primary site of glucose metabolism and FA oxidation. The insulin-sensitive C_2C_{12} myotubes were used extensively to model FFA-induced skeletal muscle IR (*23*). In this study, we established the IR model by treating C_2C_{12} myotubes with palmitate (*4*, *23*, *24*). The results of a glucose uptake assay showed that c9,t11-CLA-induced elevation of insulin-stimulated glucose uptake was dose-dependent. Even in the absence of insulin, glucose uptake was increased by c9,t11-CLA. In addition, we found that c9,t11-CLA inhibited the activity of GS, down-regulated the expression of PDK4, increased the levels of molecular markers of FA oxidation, and activated AMPK directly in the IR model. Finally, our results revealed for the first time that the increased consumption of glucose and FA acted concurrently in the IR-attenuating effects of c9,t11-CLA through the activation of AMPK.

IR is a metabolic disorder status that leads to the impairment of glucose utilization. Medicines that enhance insulin sensitivity can make a marked improvement of the impaired glucose disposal. Apart from increasing insulin sensitivity, another desirable pathway that simultaneously enhances glucose and FA consumption of insulin signaling independently can also potentially improve glucose utilization (*10*). Interestingly, our data demonstrated that c9,t11-CLA could promote glucose utilization independent of insulin. In support of this concept, we found that c9,t11-CLA increased both the insulin-stimulated and basal glucose uptake and did not affect the Akt, which is an important factor involved in the insulin signaling pathway. In addition, we found that under both conditions (+ or − insulin), the activity of GS was down-regulated after treatment with c9,t11-CLA, and thus the part of glycogen synthesis mediated by GS was attenuated. Because GS is a rate-limiting enzyme in glycogen synthesis, we believe that the anabolism of glucose was probably weakened due to the inhibition of GS by c9,t11-CLA. We also observed that c9,t11-CLA decreased the level of PDK4. We know that PDK phosphorylates and inactivates PDC, which catalyzes a rate-limiting step of glucose oxidation, that is, the conversion of pyruvate to acetyl CoA (*25*). Therefore, the increased PDK4 expression would inhibit glucose oxidation (*26*). We found that c9,t11-CLA reduced the level of PDK4 in IR myotubes, and this result suggested that the glucose aerobic oxidation was enhanced. Therefore, the increased glucose uptake, the decreased glycogen synthesis, and the enhanced aerobic oxidation together suggested that c9,t11-CLA increased catabolism of glucose.

In FA metabolism, CPT-1 controls the transfer of long-chain fatty acyl CoA into mitochondria and is the rate-limiting enzyme in FA β-oxidation. ACC mediates the catalytic formation of malonyl CoA from acyl CoA in FA synthesis. Malonyl CoA is both an intermediate in the de novo synthesis of long-chain fatty acids (LCFA) and an inhibitor of CPT1 (*11*). The decrease of p-ACC, which is the inactive form of the enzyme, leads to an increase in malonyl CoA content, thus enhancing its inhibitory activity on CPT-1. The decline of CPT-1 activity contributes, at least partly, to the decrease of FA oxidation in IR. This concept is supported by earlier studies that showed an increased content of malonyl-CoA in animal models of obesity-induced IR (*1*, *27*) and our data showing a consistent decrease of p-ACC and CPT-1 in the IR models. In addition, we found that p-ACC and CPT-1 were up-regulated by c9,t11-CLA under basal conditions in the IR model and that c9,t11-CLA had similar effects on CPT-1 whether there was insulin or not. Therefore, we believe that the treatment of c9,t11-CLA probably reversed the disorder of FA oxidation.

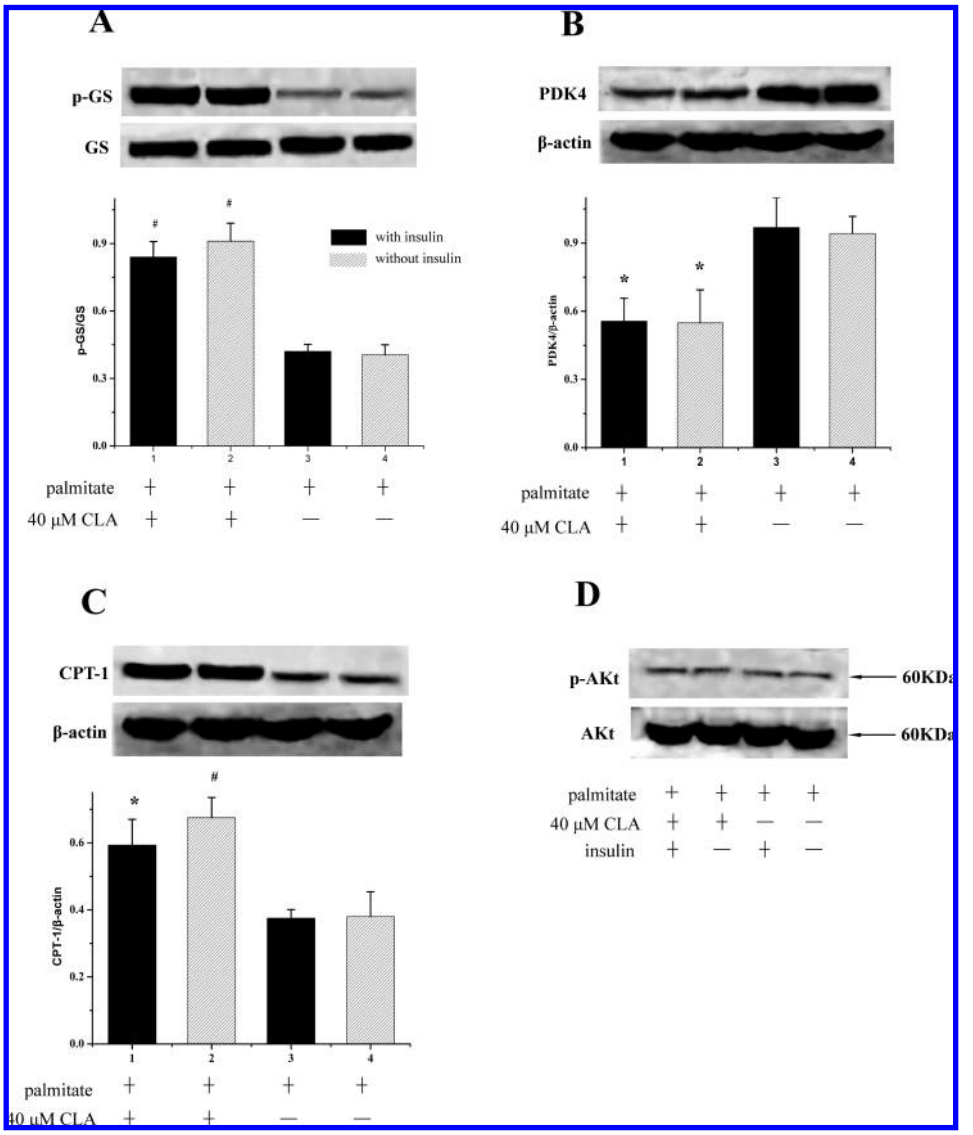

Figure 4. Effects of c9,t11-CLA on glucose and FA metabolism in IR myotubes with or without insulin. The myotubes were incubated in 0.25 mM palmitate and treated with 40 μM c9,t11-CLA (lanes 1 and 2) or without c9,t11-CLA (lanes 3 and 4) in the presence (lanes 1 and 3) or absence (lanes 2 and 4) of 100 nM insulin for 18 h. (**A**) Levels of p-GS and GS were quantified by densitometry, and values of p-GS relative to GS are expressed in the bar chart. (**B**) Levels of PDK4 and β-actin were quantified by densitometry, and values of PDK4 relative to β-actin are expressed in the bar chart. (**C**) Levels of CPT-1 and β-actin were quantified by densitometry, and values of CPT-1 relative to β-actin are expressed in the bar chart. (**D**) Levels of p-Akt and Akt were quantified. #, <0.01, and $*$, $P < 0.05$ versus myotubes treated without CLA (lanes 3 and 4). Data are expressed as mean $\pm$ SD of three independent experiments.

Because glucose catabolism and FA oxidation were promoted, it was likely that the glucose and FA consumption were increased simultaneously by c9,t11-CLA. Nevertheless, according to the glucose−FFA cycle theory of Randle, the oxidations of glucose and FA compete with each other in energy metabolism. As far as we know, there are probably several potential intracellular mechanisms in addition to the original glucose−FFA cycle theory (*28*), and we hypothesize that there was a key molecule responsible for the observed effects of c9,t11-CLA on the consumption of glucose and FA.

AMPK is a heterotrimeric protein that consists of three major subunits: a catalytic α-subunit, a regulatory β-subunit, and a γ-subunit. This enzyme can be activated allosterically by decreases in the energy state (*11*) or by the phosphorylation of Thr172 within the activation loop of the α-subunit domain directly by upstream kinases (*29*). Activated AMPK phosphorylates ACC at Ser79 and inhibits its activation (*11*), and it acts on GLUT1 and GLUT4 and increases glucose uptake (*30*). Thus, AMPK is considered to be a master switch in the regulation of glucose and FA metabolism (*11*). AMPK can stimulate glucose and lipid oxidation simultaneously to produce energy while turning off energy-consuming processes, including glucose and lipid production, to restore energy balance (*10*). We therefore propose that AMPK was likely to be targeted by c9,t11-CLA. Thus, it would be interesting to examine the level of AMPK across the treatments of c9,t11-CLA. In the present study, we observed the inhibitory effect of palmitate on AMPK, which was in accordance with the theory of Martin et al. that diet-induced obesity decreased AMPK activity in skeletal muscle (*31*). We found that the level of p-AMPK was significantly higher after treatment of c9,t11-CLA (**Figure 5**), and it was very likely that c9,t11-CLA acted on the α-subunit but not on the β-subunit. However, we still needed to examine the upstream kinases of AMPK to exclude the possibility that they activated AMPK. Two upstream kinases of AMPK have been identified—LKB1 (*32*) and calmodulin kinase kinase (CaMKK) (*33*). CaMKK is abundant in the central nervous system, but rarely expressed in other tissues (*10*). As a result, we determined the level of LKB1; neither the level of LKB1

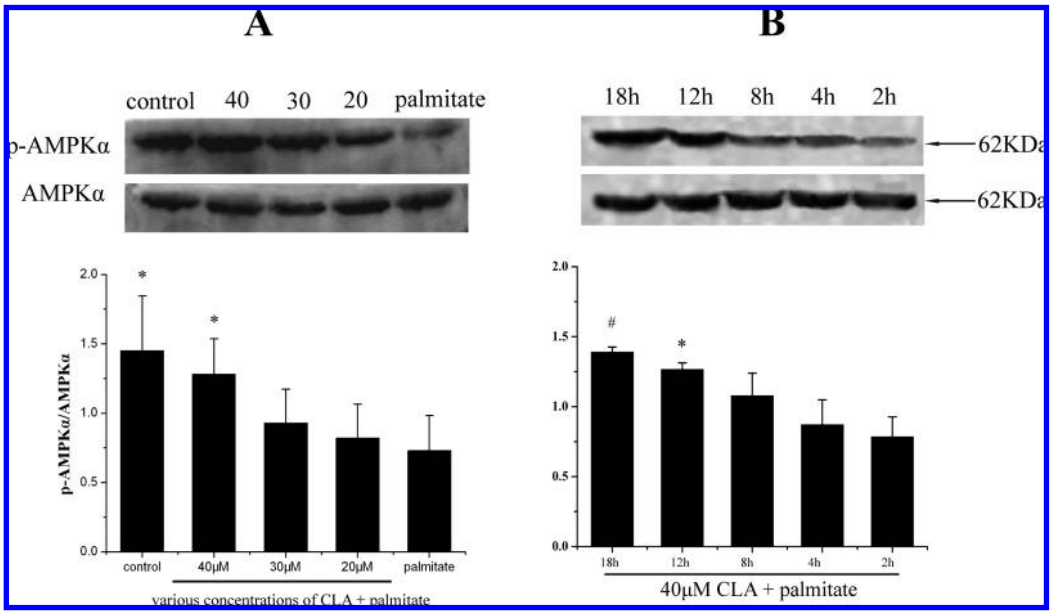

Figure 5. Effect of c9,t11-CLA on AMPK in IR myotubes. (**A**) Treatments of each group were performed as described for **Figure 2**. (**B**) The myotubes were co-incubated with 0.25 mM palmitate and 40 μM c9,t11-CLA for 2, 4, 8, 12, and 18 h, respectively. The levels of p-AMPKα and AMPKα were quantified by densitometry, and AMPK activity is expressed as the value of p-AMPKα relative to AMPKα. (**A**) $*$, $P < 0.05$ versus myotubes incubated with palmitate only. (**B**) #, <0.01, and $*$, $P < 0.05$ versus myotubes co-incubated with palmitate and CLA for 2 h. Data are expressed as mean ± SD of three independent experiments.

nor the ratio p-LKB1/LKB1 was altered significantly across treatments. Therefore, our data support the hypothesis that AMPK was targeted directly by c9,t11-CLA and demonstrate that c9,t11-CLA reversed the inhibitory effect of palmitate on AMPK by phosphorylating the α-subunit.

Future work should be focused on providing direct evidence relating to the glucose consumption and LCFA oxidation in the cell model, and animal experiments are also required for further verification of the efficacy of c9,t11-CLA on energy metabolism in other tissues and in vivo.

In conclusion, this study reported the novel finding that c9,t11-CLA increased insulin sensitivity and enhanced glucose and FA consumption independent of insulin, and data from our study suggested that AMPK was likely to be the molecular target correlated with the IR-attenuating activity of c9,t11-CLA. Therefore, it is a potential strategy to attenuate obesity-induced IR through nutrient-based activation of the AMPK pathway.

ABBREVIATIONS USED

c9,t11-CLA, *cis*-9,*trans*-11-conjugated linoleic acid; AMPK, AMP-activated protein kinase; IR, insulin resistance; FFA, free fatty acids; FA, fatty acid; GS, glycogen synthase; ACC, acetyl CoA carboxylase; PDK4, pyruvate dehydrogenase kinase 4; CPT-1, carnitine palmitoyltransferase-1; PDC, pyruvate dehydrogenase complex; Akt, protein kinase B/Akt; LCFA, long-chain fatty acids; DMSO, dimethyl sulfoxide; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; TBST, Tris-buffered saline with Tween 20; PVDF, polyvinylidene difluoride; [2-^{3}H]-DG, deoxy-D-glucose, 2-[1,2-^{3}H(N)]; 2-DG, 2-deoxy-D-glucose.

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