Involvement of AMP-Activated Protein Kinase in Glucose Uptake Stimulated by the Globular Domain of Adiponectin in Primary Rat Adipocytes

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Adiponectin is an abundant adipocyte-derived plasma protein with anti-atherosclerotic and insulin-sensitizing properties that suppresses hepatic glucose production and enhances glucose uptake into skeletal muscle. To characterize the potential effects of adiponectin on glucose uptake into adipose cells, we incubated isolated epididymal rat adipocytes with the globular domain of recombinant adiponectin purified from an E. coli expression system. Globular adiponectin increased glucose uptake in adipocytes without stimulating tyrosine phosphorylation of the insulin receptor or insulin receptor substrate-1, and without enhancing phosphorylation of Akt on Ser-473. Globular adiponectin further enhanced insulin-stimulated glucose uptake at submaximal insulin concentrations and reversed the inhibitory effect of tumor necrosis factor-α on insulin-stimulated glucose uptake. Cellular treatment with globular adiponectin increased the Thr-172 phosphorylation and catalytic activity of AMP-activated protein kinase and enhanced the Ser-79 phosphorylation of acetyl CoA carboxylase, an enzyme downstream of AMP kinase in adipocytes. Inhibition of AMP kinase activation using carboxylase, an enzyme downstream of AMP kinase in enhanced cellular fat oxidation (12) and appear to involve AMP-activated protein kinase (AMP kinase), a widely expressed serine kinase responsive to hypoxia and cellular stress that has been strongly implicated in a variety of pleiotropic cellular responses, including the insulin-independent activation of glucose transport in skeletal muscle (23).

The reduction in plasma adiponectin levels in visceral obesity is in sharp contrast to the increased secretion of cytokines such as tumor necrosis factor-α (TNF-α) in these pathological situations (24,25). TNF-α has been shown to have direct negative effects on insulin-signaling pathways in its target cells (26–29). In vascular cells, adiponectin can oppose various adverse effects of TNF-α; this observation has been especially well documented in animal models (6). Thus, a better understanding of the cellular mechanism of adiponectin’s action may provide...
insight into signaling abnormalities that occur in patients with obesity and type 2 diabetes as well as in vascular tissues.

In the present study, we explored whether the global domain of adiponectin has effects on glucose uptake in isolated adipocytes. Our results indicate that globular adiponectin increases basal glucose uptake in adipocytes and enhances insulin-stimulated glucose uptake at submaximal insulin concentrations. Moreover, globular adiponectin reverses the inhibitory effect of TNF-α on insulin-stimulated glucose uptake. We also showed that AMP kinase is involved in the stimulation of glucose uptake by globular adiponectin in mature adipose cells, as inhibitors of AMP kinase activity abrogate the increase in glucose uptake elicited by globular adiponectin. These findings provide an important mechanistic link implicating AMP kinase in the signaling effects of adiponectin in diverse, metabolically responsive tissues.

RESEARCH DESIGN AND METHODS
Materials. General reagents were of the highest available grade and were obtained from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). The pTrcHisA vector was obtained from Invitrogen (Carlsbad, CA). The nickel ion-agarose affinity column was from Qiagen (Valencia, CA). The Acticlean Etox column was from Sterogene Bioseparations (Carlsbad, CA), nickel ion-agarose affinity column was from QIAGEN (Valencia, CA). The –D-ribofuranoside (AICAR) was obtained from Toronto Research Chemicals (North York, Ontario). Protein A agarose beads were from Amersham Biosciences (Piscataway, NJ). All antibodies were polyclonal rabbit antisera, except as otherwise indicated. Anti–insulin receptor (β-subunit) and anti–insulin receptor substrate-1 (IRS-1) antibodies and anti-phosphorytine monoclonal antibodies (clone 4G10) were obtained from Upstate Biotechnology (Lake Placid, NY). Phospho-Akt Ser-473 antibody and pan-Akt isoform protein antibody were from Cell Signaling Technology (Beverly, MA). The phospho-AMP kinase Thr-172 and AMP kinase α1 + α2 isoform antibodies were from Cell Signaling Technology. The AMP kinase α2 isoform antibody, phospho-acetyl CoA carboxylase (ACC) Ser-79 antibody and anti-phosphotyrosine monoclonal antibody were from Cell Signaling Technology (Beverly, MA). The phospho-AMP kinase Thr-172 and AMP kinase α1 + α2 isoform antibodies were from Cell Signaling Technology. The AMP kinase α2 isoform antibody, phospho-acetyl CoA carboxylase (ACC) Ser-79 antibody and SAMs peptide were from Upstate Biotechnology. Compound C (6-[1-(2-piperidin-1-yl-ethoxy)-phenyl]-3-pyridin-4-yl-pyrazolo[1,5-a] pyrimidine), a piperidin-1-yl-ethoxy)-phenyl)-3-pyridin-4-yl-pyrazolo[1,5-a] pyrimidine), a potential and selective small-molecule AMP kinase inhibitor (30), was generously provided by Merck Research Laboratories (Rahway, NJ). Adenine 9-β-D-arabinofuranoside (araA) was obtained from Sigma, and 5-aminomidoze-4-carboxamide-1-β-D-arabinofuranoside (AICAR) was obtained from Toronto Research Chemicals (North York, Ontario). Protein A agarose beads were obtained from Pierce (Rockford, IL). Secondary antibodies and reagents for enhanced chemiluminescence were obtained from Perkin-Elmer Life Sciences (Boston, MA).

Animals. Male SD rats weighing 220–240 g were obtained from Charles River Laboratories (Wilmington, MA). The animal handling and euthanasia protocol was approved by the Institutional Animal Care and Use Oversight Committee of Thomas Jefferson University.

Preparation of the recombinant globular domain of adiponectin. The cDNA encoding the full-length adiponectin sequence (31) was obtained by PCR from human adipose tissue RNA, and the truncated globular domain (amino acids 108–244) was subcloned into the pTrcHisA bacterial expression vector. The recombinant adiponectin globular domain was expressed as an N-terminal 6 × His-tagged fusion protein in E. coli DH5α, by induction with isopropyl β-D-thiogalactopyranoside. bacterial cell lysates were prepared under native conditions, and the recombinant globular adiponectin was isolated over nickel-ion-agarose affinity column according to the manufacturer’s instructions (Qiagen). After elution, the protein was applied to an Acticlean Etox column to remove potential endotoxin contamination, the absence of which was verified by limulus amebocyte lysate assay.

Isolation of rat adipocytes. Rat adipocytes were isolated as previously described (32). Briefly, excised rat epididymal adipose tissue was handled under sterile conditions and rinsed in warm Hank’s balanced salt solution in polypropylene tubes (Falcon 2050). Visible blood vessels were removed, and the fat pads were minced into millimeter-size pieces. The adipose tissue was then digested by Krebs-Ringer bicarbonate HEPES (KRH) buffer (120 mmol/l NaCl, 4 mmol/l KH2PO4, 1 mmol/l MgSO4, 0.75 mmol/l CaCl2, 10 mmol/l NaHCO3, 30 mmol/l HEPES; pH 7.4) with 1 mg/ml collagenase type I, 1% (wt/vol) BSA, 2.5 mmol/l glucose, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 1% (vol/vol) fungizone for 40–60 min in a 37°C water bath with gentle agitation. After being filtered sequentially through 500- and 250-μm nylon mesh, the adipocyte cell suspension was centrifuged at 800 g at room temperature for 2 min. Culture media were washed twice with warm KRH buffer containing 1% BSA, 2.5 mmol/l glucose, and antibiotics.

Adipocyte treatments. For the glucose uptake assay, cells were washed two times in KRH buffer (pH 7.4) with 1% BSA but without glucose; for the AMP kinase phosphorylation assay, cells were washed two times in KRH buffer (pH 7.4) with 1% BSA containing 2.5 mmol/l glucose. Cells were then resuspended in the wash buffer to 20% of the initial density. Redistribution of cell lysates, the adipose cells were treated at 37°C with the indicated concentrations of globular adiponectin for 2 h, AICAR for 20 min, insulin for 5 min, or TNF-α for 20 min. Where indicated, cell treatment with araA or compound C was initiated 20 min before the addition of globular adiponectin. Measurement of [14C]glucose and 2-deoxy-D-[14C]glucose uptake. Glucose uptake was measured as previously described (32). Adipocyte suspensions (150–210 μl) were equilibrated in triplicate in polypropylene vials for 10 min at 37°C and uncapped with gentle agitation, KRH buffer (50 μl) with or without insulin was then added and the incubation proceeded for 5–30 min at 37°C, as indicated. Next [14C]glucose (1 μCi) or 2-deoxy-D-[14C]glucose (0.5 μCi) was added to each vial for 5 or 2 min, respectively, and the transport reaction was terminated by the addition of cytochalasin B (10 μl of a 1.5 mmol/l solution). The adipocyte suspension was then pipetted into a 450-μl vial containing theelinophylline (100 μmol/l) and centrifuged at 2 min for 6,000 g at room temperature. The tubes were cut through the oil layer, the tube fragments containing the packed cells were dropped into polyethylene vials containing 4 ml liquid scintillation fluid, and the cell-associated radioactivity was measured in a β-counter. The level of nonspecific radioactivity in the cell pellet was determined by adding cytochalasin B to a set of control samples before incubation with [14C]glucose or 2-deoxy-D-[14C]glucose. These background counts were subtracted from all the values, and after the cell concentration was determined, the glucose uptake was normalized by cell number, as described by Digrilano et al. (33).

Immunoblotting for protein phosphotyrosine levels, phospho-Akt, phospho-AMP kinase, and phospho-ACC. After treatment of the adipocytes, the incubation buffer was removed. The cells were then washed twice in KRH containing 2.5 mmol/l glucose and disrupted into 1 ml lysis buffer (50 mmol/l HEPES [pH 7.4], 150 mmol/l NaCl, 1% (vol/vol) Triton X-100, 5 mmol/l EDTA, 5 mmol/l EGTA, 20 mmol/l Na pyrophosphate, 20 mmol/l NaF, 1 mmol/l Na orthovanadate, 1 mmol/l β-glycerophosphate, 1 mmol/l phenylmethylsulfonyl fluoride, and protease inhibitor cocktail). The cell lysate was sonicated twice for 10 s each on ice and centrifuged at 2,000 rpm for 10 min and 12,000 rpm for 20 min at 4°C to gradually remove the fat layer. The lysate supernatant was then isolated by centrifugation at 100,000g for 1 h and the protein concentration was estimated using Bio-Rad protein dye reagent, as described by the manufacturer.

For evaluation of insulin receptor and IRS-1 phosphotyrosine levels, lysates from the treated adipocytes containing 200 μg of protein were first incubated with 1.0 μg insulin receptor or IRS-1 antibody overnight at 4°C, then incubated with 40 μl protein A agarose beads for 2 h at 4°C. The agarose was pelleted by centrifugation at 2,000 rpm for 2 min and washed four times in cell lysis buffer. The agarose beads were mixed with 20 μl SDS sample protein buffer and boiled at 100°C for 5 min. After being centrifuged at 4,000 rpm for 2 min, the supernatant was resolved by SDS-PAGE (7.5% wt/vol) and transferred to polyvinylidene fluoride (PVDF) membrane. Primary blotting antibodies were used according to the manufacturer’s instructions. Visualization of bound antibodies was performed by incubation with horseradish peroxidase–conjugated secondary antibodies and then enhanced chemiluminescence, exposure to X-ray film, and quantification using the Image Station 440CF (Kodak, Rochester, NY). To normalize the blots for protein levels, after being immunoblotted with anti-phosphospecific antibodies, the blots were treated for 20 min at 37°C using a stripping buffer solution (Pierce), washed several times with Tris-buffered saline with Tween-20 (0.05% vol/vol), and reprobed using polyclonal antibodies to Akt, AMP kinase, or ACC.

Immunoprecipitation and AMP kinase enzyme assay. Cell lysates were prepared as described for immunoblotting. Aliquots containing 200 μg of lysate were incubated with 1.0 μg anti-AMP kinase α2 polyclonal rabbit antibody overnight at 4°C, then incubated with 40 μl protein A agarose beads for 2 h at 4°C. The agarose was washed four times using AMP kinase reaction buffer (20 mmol/l HEPES-NaOH [pH 7.0], 0.4 mmol/l dithiothreitol with 300 μmol/l AMP) and then incubated with 20 μl AMP kinase reaction buffer, 10 μl SAM substrates peptide (80 μmol/l ATP, 125 μmol/l MgCl2, 30 mmol/l magnesium chloride and 500 μmol unlabeled ATP for 15 min at 30°C. Aliquots of 35 μl were spotted onto the center of a 2-cm square of Whatman P81 paper.
The P81 squares were washed three times with 0.75% (vol/vol) phosphoric acid and then transferred to a vial containing 5 ml of scintillation cocktail. The radioactivity was then counted in a β-counter. AMP kinase activity was expressed as the phosphorylation of SAMS peptide (dpm per microgram protein per minute).

Statistical analyses. Quantitative data are expressed as means ± SE from at least three replicate determinations. Student’s t test was used for comparing two group means, and ANOVA was used for multiple comparisons. Differences were regarded as significant at P < 0.05.

RESULTS

Globular domain of adiponectin enhances glucose uptake into mature rat adipocytes. The effect of globular adiponectin on glucose uptake in mature rat adipose cells was evaluated using both D-[14C]glucose and 2-deoxy-D-[3H]glucose (Fig. 1). Treatment with the adiponectin globular domain alone for 2 h at concentrations of 100–800 ng/ml increased D-glucose uptake by 50–100% (all P < 0.001 vs. control). Stimulation with insulin alone for 5 min at a submaximal concentration (35 nmol/l) increased D-glucose uptake by 2.4-fold (P < 0.001). D-glucose uptake was further increased by co-stimulation with insulin and globular adiponectin, by up to 34% compared to insulin alone with 800 ng/ml globular adiponectin (P = 0.002). Using the 2-deoxy-D-glucose assay (Fig. 1B), globular adiponectin alone at 500 and 1,000 ng/ml enhanced deoxyglucose uptake by 57 and 89%, respectively (both P < 0.001). Insulin (35 nmol/l) stimulated the uptake of 2-deoxy-D-glucose by 2.3-fold (P < 0.001). Stimulation with insulin and globular adiponectin increased the uptake of deoxyglucose by up to 82%, compared to samples with no insulin stimulation (P < 0.001). These data established that globular adiponectin increased basal glucose uptake and significantly enhanced insulin-stimulated glucose uptake in a dosage-dependent manner.

To evaluate the time course of global adiponectin–stimulated glucose uptake, rat adipocytes were incubated with 1,000 ng/ml globular adiponectin for up to 3 h. The uptake of 2-deoxy-D-glucose was significantly increased by fivefold after treatment with globular adiponectin for 30 min, and the increase was sustained for at least an additional 2 h (Fig. 2).

Adiponectin globular domain reverses the inhibition of basal or insulin-stimulated glucose uptake by TNF-α in rat adipocytes. To determine whether globular adiponectin could reverse the inhibition of adipocyte glucose transport mediated by TNF-α, adipose cells were incubated with 500 ng/ml globular adiponectin for 1.5 h after treatment with or without an inhibitory concentration of 20 ng/ml TNF-α for 20 min (34), followed by 35 nmol/l insulin for the last 5 min of the cell incubation before measurement of 2-deoxy-D-glucose uptake (Fig. 3). TNF-α attenuated the stimulation of glucose uptake by globular adiponectin, insulin, and the two agents together by 45.0, 21.0, and 16.7%, respectively (P < 0.01). Compared to samples treated with TNF-α alone or TNF-α with insulin, stimulation by globular adiponectin enhanced glucose uptake by 89 and 31%, respectively (both P < 0.01), effectively reversing the suppression of glucose uptake by TNF-α (Fig. 3).

Adiponectin globular domain does not affect insulin-stimulated tyrosine phosphorylation of the insulin receptor or IRS-1 or serine phosphorylation of Akt in mature rat adipocytes. To evaluate the signaling pathways used by globular adiponectin in the adipose cells, we first tested whether globular adiponectin enhanced the level of tyrosine phosphorylation of the insulin receptor or IRS-1. Cellular stimulation under conditions identical to those used to enhance glucose transport noted above significantly increased the tyrosine phosphorylation of the
insulin receptor and IRS-1 in immunoprecipitated cell lysates (Fig. 4). In contrast, globular adiponectin treatment of the adipocytes had no effect on insulin receptor and IRS-1 tyrosine phosphorylation. Similarly, the downstream activation of Akt was significantly enhanced by insulin under these conditions, whereas globular adiponectin had no effect on Akt Ser-473 phosphorylation (Fig. 4).

Adiponectin globular domain stimulates phosphorylation and activity of AMP-activated protein kinase in rat adipocytes. To evaluate the potential role of AMP kinase in the cellular effects of globular adiponectin in rat adipocytes, lysates from cells incubated with globular adiponectin were blotted with phospho-AMP kinase (Thr-172) antibody (Fig. 5A). As quantitated in Fig. 5B, over the dosage range of 250–1,000 ng/ml, globular adiponectin increased the phosphorylation of AMP kinase up to twofold.

The time course of globular adiponectin–induced AMP kinase phosphorylation was also evaluated (Fig. 6). Using 1,000 ng/ml globular adiponectin, the phosphorylation of AMP kinase was significantly increased starting at 30 min of incubation. A saturating level of activation was reached at an increase of ~1.8-fold by 2–3 h. The overall time course of increased AMP kinase phosphorylation was significantly delayed compared to insulin activation of the insulin signaling pathway (Fig. 4) and, interestingly, preceded the activation of glucose uptake by globular adiponectin, which did not appear until after 30 min of stimulation (Fig. 2).

In control experiments, cells treated with the AMP kinase activator AICAR alone increased AMP kinase phosphorylation more than twofold compared to controls (Fig. 5B). Using samples of cell lysates immunoprecipitated with the AMP kinase α2 subunit antibody, globular adiponectin (250–1,000 ng/ml) was also shown to significantly increase the activity of AMP kinase by up to 47% ($P < 0.001$) (Fig. 7A). Similarly, the immunoprecipitates of
AMP kinase α1 + α2 showed increased activity by up to 24% (Fig. 7B).

araA, a metabolic precursor of araATP, is an intracellular competitive inhibitor of AMP kinase and blocks AMP kinase–related glucose uptake in skeletal muscle (35,36). Treatment of the cells with araA had no effect on basal AMP kinase (Thr-172) phosphorylation or enzyme activity in α2 or α1 + α2 isoform immunoprecipitates (Figs. 5 and 7). However, araA significantly decreased AMP kinase phosphorylation stimulated by globular adiponectin treatment as well as by globular adiponectin and AICAR (Fig. 5). Importantly, araA fully suppressed the enzyme activity of AMP kinase α2 and α1 + α2 isoforms stimulated by globular adiponectin (Fig. 7). Less of an effect was noted on araA suppression of α2 isoform activity in immunoprecipitates from cells also treated with AICAR (Fig. 7A).

**Adiponectin globular domain enhances the phosphorylation of acetyl CoA carboxylase.** ACC is the rate-controlling enzyme downstream of AMP kinase for fatty acid synthesis and generation of intracellular malonyl-CoA, which regulates fatty acid oxidation (37). Consistent with the upstream activation of AMP kinase discussed above, treatment of adipocytes with globular adiponectin (250–1,000 ng/ml) also enhanced the phosphorylation (Ser-79) of ACC by up to 86% compared to the basal level (P < 0.001) (Fig. 8). AICAR treatment also increased ACC phosphorylation by more than twofold, whereas araA decreased the basal phosphorylation of ACC by 20%. Consistent with the inhibitory effect of araA on AMP kinase activation by globular adiponectin treatment, araA also dramatically suppressed the level of ACC phosphorylation stimulated by globular adiponectin.

**Inhibition of AMP kinase activation blocks glucose uptake stimulated by the adiponectin globular domain but not by insulin.** Two inhibitors of AMP kinase were used to characterize the role of this enzyme in globular adiponectin–stimulated glucose uptake: araA and compound C. Adipocytes were preincubated with or without 2 μmol/l araA or 10 μmol/l compound C for 20 min after treatment with 500 ng/ml adiponectin globular domain for 2 h; then uptake of 2-deoxy-D-[3H]glucose was measured (Fig. 9). Where indicated, cells were also stimulated with or without insulin (35 nmol/l) for 5 min before assay of glucose uptake. Adiponectin globular domain alone, insulin alone, and globular adiponectin plus insulin enhanced the uptake of 2-deoxyglucose by 2.1-, 2.3-, and 2.6-fold, respectively (P < 0.001). Treatment of cells with araA or compound C had no effect on the level of basal or insulin-stimulated glucose uptake. However, both araA and compound C completely suppressed the level of glucose uptake stimulated by globular adiponectin.

**DISCUSSION**

Over the past few years, our understanding of adiponectin as a potential key regulator of glucose and fat homeostasis as well as of vascular function in states of obesity and insulin resistance has grown dramatically (38,39). More
recently, insight has been gained into some of the properties of this protein in cellular signal transduction mechanisms, particularly in skeletal muscle and liver tissue; however, previous reports have not examined adiponectin action in adipose cells. In the present work, we demonstrated that the adiponectin globular domain alone has significant effects on glucose uptake in adipose tissue and that globular adiponectin also enhances insulin-stimulated glucose uptake and opposes TNF-α–inhibited glucose uptake in isolated adipocytes. Interestingly, the mechanism of global adiponectin action in these cells does not involve changes in the tyrosine phosphorylation of key regulatory proteins in the insulin action pathway, but does involve activation of AMP kinase, providing a link between adiponectin signaling in skeletal muscle and liver with adipose tissue.

In contrast to our data in adipose tissue, adiponectin has recently been shown to enhance insulin-stimulated receptor tyrosine phosphorylation in skeletal muscle in animals and human subjects. Adiponectin is positively associated with insulin sensitivity and receptor tyrosine phosphorylation in skeletal muscle in human subjects (40). Administration of the adiponectin globular domain to lipoatrophic mice deficient in circulating adiponectin also potentiates insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1 and enhanced Akt activation in skeletal muscle (16). These findings suggest that the mechanism of some of the regulatory effects of adiponectin on cellular signaling may be different between adipose and skeletal muscle tissue. Furthermore, because our data were obtained by directly treating isolated adipocytes with the globular domain of adiponectin in vitro, it is possible that some of the effects observed with adiponectin on skeletal muscle in vivo were indirect.

In isolated adipose cells, globular adiponectin increases the threonine phosphorylation of AMP kinase, activates AMP kinase enzyme activity, and enhances the phosphorylation of ACC, a metabolic enzyme known to be downstream of AMP kinase (37). Moreover, we found that globular adiponectin–induced glucose uptake can be blocked by inhibitors of AMP kinase, indicating that AMP kinase activation mediates the effects of globular adiponectin on glucose uptake. This is in contrast to insulin-stimulated glucose uptake, which is not affected by the inhibitors of AMP kinase. AMP kinase is a highly conserved heterotrimeric signaling kinase responsive to hyp-
oxia, exercise, and cellular stress that has been strongly implicated in a variety of cellular responses, including suppression of gluconeogenesis in the liver, promotion of glucose uptake in skeletal muscle, inhibition of fatty acid and sterol synthesis, increases in fatty acid oxidation, and inhibition of lipolysis (23,37,41–43). The α2 isoform of the catalytic subunit is predominant in liver, skeletal muscle, and endothelial cells (44,45); however, both subunits are reportedly expressed in 3T3-L1 adipocytes (46,47).

Only a few previous studies have focused on the potential role of AMP kinase in glucose uptake in adipose cells. The use of the AMP kinase activator AICAR in attempts to delineate a role of AMP kinase in glucose transport activation in adipocyte models, has generated inconclusive results. AICAR increases AMP kinase activity and enhances glucose uptake in muscle tissue (36,48). Although treatment of differentiated 3T3-L1 adipocytes with AICAR reportedly enhanced glucose transport by up to twofold, overexpression of a dominant negative AMP kinase α2 construct abolished AMP kinase activation without affecting glucose transport, raising questions about whether AMP kinase is directly involved in AICAR-stimulated glucose transport in this adipocyte model (47,49). In the primary adipocytes, we also found that AICAR significantly increased AMP kinase phosphorylation at Thr-172 (Fig. 5), but did not increase AMP kinase enzyme activity (Fig. 7) or glucose uptake (data not shown) to a similar degree. As an intermediate in de novo purine biosynthesis, AICAR may exert additional cellular effects and may not be specific for AMP kinase activation (50). This may potentially explain why AICAR has little effect on adipocyte glucose uptake. Also, globular adiponectin may exert effects through additional pathways that may influence the ultimate stimulation of glucose uptake by globular adiponectin in addition to its enhancement of AMP kinase activity.

Studies in other cellular systems (including undifferentiated 3T3-L1 cells, C2C12 myoblasts, and clone 9 cells) have also implicated AMP kinase in GLUT1-mediated glucose transport, including actual activation of GLUT1 transporters preexisting in the plasma membrane (51,52). The mechanism(s) by which AMP kinase enhances cellular glucose transport has been best studied in skeletal muscle, where it has been linked to changes in GLUT4 transcription and translocation (41,48,53). Clearly, further work in this area may help to distinguish AMP kinase signaling mechanisms involving glucose uptake in muscle and adipose tissue.

The potent effect of araA and compound C in blocking globular adiponectin–stimulated glucose uptake strongly suggests a role for AMP kinase in the signaling pathway for insulin-independent glucose uptake stimulated by globular adiponectin in adipose cells. Our data demonstrating that araA decreases globular adiponectin–stimulated AMP kinase activation and ACC phosphorylation also provide further evidence in support of previous work showing that araA serves as an effective inhibitor of AMP kinase (35,36). The use of these inhibitors also helps to differentiate the mechanism of the globular adiponectin effect from that of insulin-stimulated glucose uptake, which was not affected by the AMP kinase inhibitors. Recently, AMP kinase has also been implicated in the action of adiponectin in skeletal muscle and liver tissue. Yamauchi et al. (22) first reported that AMP kinase is activated in mouse skeletal muscle by treatment in vivo with either the globular domain or full-length adiponectin, and that AMP kinase activation mediates the stimulation of glucose utilization and fatty acid oxidation by adiponectin. Interestingly, in this work, only full-length adiponectin was active in the liver to stimulate AMP kinase and metabolic signaling (22). Tomas et al. (21) have also reported that treatment of fast-twitch skeletal muscle with the globular domain of adiponectin increased AMP kinase activity twofold within 30 min, which was also associated with increased serine phosphorylation of ACC and a 1.5-fold increase in 2-deoxyglucose uptake. In contrast, the full-length hexameric form of adiponectin did not activate AMP kinase or ACC in fast-twitch muscle, and the globular adiponectin domain did not affect AMP kinase or glucose transport in slow-twitch muscle. Clearly, additional work will be necessary to further characterize whether the various molecular forms of adiponectin differ in their effects on AMP kinase activation or downstream signaling in these major tissues responsible for metabolic regulation.

The action of globular adiponectin in adipocytes also reverses the inhibitory effect of TNF-α on insulin-stimulated glucose uptake. The adverse effect of TNF-α has been previously shown to involve early events in insulin signaling involving insulin receptor autophosphorylation and IRS-1 tyrosine phosphorylation in fat tissue (54,55). Some of the beneficial effects of adiponectin on vascular cells appear to be mediated by inhibition of the deleterious actions of TNF-α, which can initiate an inflammatory cascade in obesity and insulin-resistant states (56). In cultured myocytes, TNF-α decreased and adiponectin enhanced FATP-1 mRNA, IRS-1–associated phosphatidylinositol 3-kinase activity, and glucose uptake (57). Without blocking TNF-α binding, adiponectin inhibits TNF-α–induced expression of adhesion molecules on cultured endothelial cells and TNF-α–induced endothelial adhesion of mononuclear cells (6), an action likely mediated by adiponectin suppression of TNF-α–induced IκB phosphorylation and nuclear factor-κB activation (58). Thus, adiponectin may play an important salutary role, potentially using shared signaling pathways in metabolic as well as in vascular cell types.

The finding that glucose uptake is enhanced by globular adiponectin in adipose cells is consistent with the observations that human subjects with type 2 diabetes have low circulating concentrations of adiponectin (9) and Pima Indians with high circulating concentrations of adiponectin are significantly less likely to develop hyperglycemia (59). The identification of enhanced glucose uptake in response to adiponectin in a mature primary cell type, and the elucidation of the role of the AMP kinase pathway in this process, provides a novel system for further detailed analysis of the adiponectin signal transduction cascade.

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REFERENCES


44. Flyer LGG, Foufelle F, Barnes K, Balthwin SW, Woods A, Carling D: Characterization of the role of the AMP-activated protein kinase in the