Globular adiponectin induces LKB1/AMPK-dependent glucose uptake via actin cytoskeleton remodeling

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Abstract
Previous studies have shown that many metabolic actions of adiponectin are mediated via the activation of AMP kinase and that adiponectin stimulates GLUT4 translocation and glucose uptake in the muscle. In this study, we demonstrate that adiponectin stimulates actin cytoskeleton remodeling, with increased phosphorylation of coflin, and that blocking of cytoskeletal remodeling with cytochalasin D prevents adiponectin-stimulated AMPK phosphorylation in L6 myoblasts. LKB1 is an upstream kinase of AMPK, and we observed the colocalization of LKB1 with filamentous actin in response to adiponectin. Adiponectin-stimulated translocation of LKB1 from a nuclear to a cytoplasmic location to activate AMPK was also dependent on actin cytoskeleton remodeling. Cytoskeletal remodeling visualized by rhodamine–phalloidin immunofluorescence indicated that adiponectin-stimulated reorganization resulted in the formation membrane ruffles, which were also clearly visible by scanning electron microscopy in L6-GLUT4myc myoblasts. The stimulation of glucose uptake, but not of GLUT4-myc translocation to the cell surface, by adiponectin was also dependent on actin cytoskeleton remodeling. These results suggest that actin remodeling induced by adiponectin is essential for mediating LKB1/AMPK signaling and glucose uptake in skeletal muscle cells.

Key Words
- adiponectin
- actin cytoskeleton
- LKB1
- AMPK
- glucose uptake

Introduction
The beneficial effects of adiponectin on peripheral metabolism are now well characterized (Yamauchi & Kadowaki 2008, Matsuzawa 2010, Dadson et al. 2011). Adiponectin targets multiple metabolic tissues including skeletal muscle (Ceddia et al. 2005, Satoh et al. 2005, Civitarese et al. 2006, Yoon et al. 2006, Vu et al. 2007, Liu et al. 2009), liver (Wang et al. 2009b, Mandal et al. 2010, Zhou et al. 2010, 2012), and pancreas (Wijesekara et al. 2010, Lee et al. 2011), and it is hoped that the therapeutic potential of targeting adiponectin can be harnessed (Shetty et al. 2009). This will require not only the characterization of the physiological effects of adiponectin, but also a detailed understanding of the cellular mechanisms by which these effects occur. It is apparent that many of the favorable metabolic effects of adiponectin are mediated via AMPK-dependent signaling.
Recent studies have also established that binding of ligands to adiponectin receptors (AdipoRs) leads to the recruitment of APPL1- and PKC-ζ-dependent nuclear export of LKB1 resulting in the activation of AMPK (Mao et al. 2006, Zhou et al. 2009, Fang et al. 2010, Xin et al. 2011).

The actin cytoskeleton is a dynamic structure important for cellular structural support, mobility, and compartmentalization of organelles and signaling molecules (Brozinick et al. 2007, Stockli et al. 2011). Specifically, insulin has been shown to stimulate actin cytoskeleton remodeling in skeletal muscle cells, which is important for GLUT4 translocation to the cell surface and glucose uptake (Brozinick et al. 2007, Stockli et al. 2011). Insulin induces the formation of lamellipodia membrane ruffles (Khayat et al. 2000, Huang et al. 2005), which have been shown to be sites for the colocalization of insulin signaling molecules as well as areas in which GLUT4-containing vesicles are inserted into the plasma membrane (Khayat et al. 2000, Tong et al. 2001, Brozinick et al. 2007, Stockli et al. 2011). Cytoskeleton disruption using agents such as cytochalasin D (CD) and latrunculin B can inhibit key steps in the insulin signaling cascade, including the activation of PI3K and Akt. Actin remodeling is a dynamic process where globular actin (G-actin) is polymerized into filamentous actin (F-actin) (Lee & Dominguez 2010). A family of Rho-GTPases (Rho, Rac, and Cdc42) regulates actin cytoskeleton dynamics through the activity of their effector proteins, Rho-associated protein kinase (ROCK) and p21-activated kinases (PAK) 1 and 4, which in turn activate LIM kinase 1 (LIMK1) via phosphorylation at threonine-508 (Lee & Dominguez 2010). Activated LIMK1 phosphorylates cofilin at serine-3, thus interfering with its filamentous actin-severing and -depolymerizing activity.

There is substantial evidence that adiponectin mediates beneficial metabolic effects, and it is now imperative to further understand the underlying mechanisms. In this study, we examined the effects of adiponectin on actin cytoskeleton remodeling in skeletal muscle cells and the subsequent impact on signaling involving LKB1 and AMPK and glucose uptake.

![Figure 1](http://jme.endocrinology-journals.org/C209)

**Figure 1**

Effect of gAd on actin cytoskeleton remodeling in L6 cells. L6-GLUT4myc myoblasts seeded onto coverslips were stimulated with 2.5 μg/ml gAd for (C) 5 to (D) 30 min, (A) control cells were unstimulated, and (B) insulin (100 nM, 10 min) served as a positive control. The F-actin network was visualized in cells stained with rhodamine-labeled phalloidin using fluorescent microscopy with a 60× oil immersion objective. Images are single 3 μm slices through the dorsal surface of the cell (bottom panel of the images (A, B, C, and D)) and collapsed ‘xy projections’ (top panel of the images in (A, B, C, and D)) representative of n ≥ 3 experiments. Full colour version of this figure available via [http://dx.doi.org/10.1530/JME-13-0059](http://dx.doi.org/10.1530/JME-13-0059).
Materials and methods

Materials

Cell culture medium (α-minimum essential medium (α-MEM), 5 mM glucose), PBS, fetal bovine serum (FBS), and antibiotic/antimycotic solutions were purchased from Wisent (St Foy, QC, Canada). Rhodamine-conjugated phalloidin, Prolong Gold antifade reagent, and Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody were obtained from Invitrogen Canada, Inc. Globular adiponectin (gAd) was produced in-house as described previously (Palanivel et al. 2007). CD, Cytochalasin B, SIGMAFAST o-phenylenediamine dihydrochloride (OPD) tablets, and 4',6-diamidino-2-phenylindole (DAPI) were obtained from Sigma–Aldrich. Human insulin (Humulin R) was obtained from Eli Lilly, and 2-deoxy-D-[3H]glucose was purchased from Amersham. Polyclonal antibodies to phosphorylated AMPK (threonine-172), phosphorylated cofilin (serine-3), β-actin, LKB1 (27D10), and HRP-conjugated anti-rabbit IgG and anti-mouse IgG were purchased from Cell Signaling Technology (Beverly, MA, USA), and polyclonal c-myc (9E10) was obtained from Santa Cruz Biotechnology, Inc. Chemiluminescence reagent plus was obtained from Perkin Elmer LAS (Boston, MA, USA), and PVDF membranes were purchased from Bio-Rad.

Cell culture

L6-GLUT4<sup>myc</sup> cells stably transfected to overexpress myc-tagged GLUT4 (a gift from Dr Amira Klip, The Hospital for Sick Children, Toronto) were used as myoblasts when grown to full confluency in α-MEM supplemented with 10% (volume/volume (v/v)) FBS and 1% (v/v) antibiotic/antimycotic solution under a humidified atmosphere of 95% air and 5% CO₂ at 37 °C.

Immunofluorescence microscopy analysis of actin cytoskeleton remodeling and GLUT4 and LKB1 localization

L6-GLUT4<sup>myc</sup> myoblasts, seeded onto 18 mm round coverslips, were serum-starved for 3 h and treated with gAd (2.5 μg/ml, 30 min), insulin (100 nM, 10 min), and/or CD (1 μM, 30 min). The cells were then washed with PBS containing 1 mM CaCl₂ and 1 mM MgSO₄ (PBS⁺) and fixed with 4% paraformaldehyde on ice for 10 min. Paraformaldehyde was quenched with 100 mM glycine (10 min at room temperature), and the cells were permeabilized with 0.01% Triton X-100 for 5 min followed by 1 h of blocking with 2% BSA and 5% goat serum at room temperature. The cells were then incubated with a primary antibody (anti-c-myc (1:200 dilution) for

Figure 2

Effect of gAd on the membrane morphology of L6 cells. Scanning electron microscopy images of L6-GLUT4<sup>myc</sup> myoblasts under (A) basal, (B) insulin-treated, and gAd (2.5 μg/ml) stimulation conditions for (D) 10 or (F) 30 min. (B) Insulin (100 nM, 10 min) was used as a positive control for membrane ruffling, while (C) CD was used to disrupt the actin cytoskeleton network.
GLUT4	ext{myc} or LKB1 (1:50 dilution)) at room temperature overnight, washed with PBS\(^{+}\), and subsequently incubated with a secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit or FITC-conjugated goat anti-mouse (1:1000 dilution for each antibody)) and rhodamine-conjugated phalloidin (0.66 nM) for 1 h at room temperature. Coverslips were washed with PBS\(^{+}\) and mounted on glass microscope slides with Prolong Gold antifade Mounting Media supplemented with 5 \(\mu\)g/ml DAPI for nuclear staining. Images were obtained using the Olympus BX51 confocal microscope (Olympus, Seattle, WA, USA) with a 60× objective under oil immersion.

**Scanning electron microscopy to visualize L6 cell surface morphology**

The cells were grown on coverslips and treated with gAd, insulin, and/or CD followed by 1 h of fixation in 2.5% glutaraldehyde at 4 °C. Fixed cells were dehydrated in a graded ethanol series (30–100%, 10 min each) and then subjected to a final drying step using two consecutive 10 min baths of hexamethyldisilazane. Coverslips were then air-dried at 37 °C for 30 s and subsequently mounted on stubs, sputter-coated (Hummer VI Au/Pd 40/60), and examined using a Hitachi S-520 scanning electron microscope. Images were captured using a passive image capture system at 6000× magnification (Hitachi, Quartz PCI Version 6).

**Glucose transport in L6-GLUT4\text{myc} myoblasts**

L6-GLUT4\text{myc} myoblasts, seeded in 24-well plates and serum-starved for 3 h before the experiments, were treated for 2 h with 2.5 \(\mu\)g/ml gAd in the absence or presence of 1 \(\mu\)M CD. In all the experiments, 100 nM insulin stimulation for 20 min was used as a positive control. Following treatments, the cells were washed twice with HEPES buffer (140 mM NaCl, 20 mM HEPES-Na, 2.5 mM MgSO\(_4\), 1 mM CaCl\(_2\), and 5 mM KCl, pH 7.4) and incubated for 5 min at room temperature in a transport solution (HEPES buffer with 0.5 \(\mu\)Ci/ml 2-deoxy-D-[\(^3\)H]glucose), and nonspecific uptake was measured in the presence of cytochalasin B (10 \(\mu\)M). The cells were lysed with 1 M KOH and transferred to scintillation vials for \(^3\)H radioactivity counting, and protein levels were determined by the Bradford protein assay. Glucose uptake was calculated as picomoles per milligram protein per minute, and it is expressed as fold change over control.

**Determination of GLUT4-myc translocation**

L6-GLUT4\text{myc} cells contain a myc epitope on the exofacial location of GLUT4, which allowed for the measurement of cell surface GLUT4 content in intact cells through the SIGMAFAST OPD antibody-coupled colorimetric assay as described previously (Ceddia et al. 2005). Following treatments, L6-GLUT4\text{myc} myoblasts were washed with ice-cold PBS and incubated for 1 h with an anti-c-myc antibody (1:200 dilution) at 4 °C. The cells were washed and fixed in 3% paraformaldehyde for 3 min on ice. The fixative was then neutralized for 10 min with 10 mmol/l glycine in ice-cold PBS. The cells were blocked in 10% goat serum and 3% BSA for 30 min and incubated for 1 h with HRP-conjugated goat anti-rabbit IgG (1:1000 dilution) at 4 °C. The cells were then washed with ice-cold PBS and incubated with the OPD reagent for 30 min at room temperature. The reaction was stopped using 3 M HCl, and the absorbance of the supernatant was measured at 492 nm.

**Figure 3**

Alteration of cofilin phosphorylation (S3) in response to gAd treatment. Phosphorylation of (A) cofilin (S3) in L6-GLUT4\text{myc} myoblasts lysates was examined following treatment with 2.5 \(\mu\)g/ml gAd; (B) graphs representing the quantitative analysis of \(n\) >3 experiments; data are expressed as means \(\pm\) S.E.M. fold above the control and \(\beta\)-actin served as a loading control. *\(P<0.05\) vs the control (Con) and #\(P<0.05\) vs CD treatment.
Immunoblot analysis of AMPK and coflin phosphorylation

Western blotting was conducted as described previously (Vu et al. 2007), where, after gAd treatment, L6-GLUT4<sub>myc</sub> myoblast lysates were lysed in 1× Laemmli sample buffer (62.5 mM Tris–HCl (pH 6.8), 2% (w/v) SDS, 50 mM dithiothreitol, 0.01% (w/v) bromophenol blue, and phosphatase inhibitors (1 μM Na<sub>3</sub>VO<sub>4</sub>, 1 μM leupeptin, 1 μM pepstatin, 1 μM okadaic acid, and 1 μM phenylmethylsulfonyl fluoride)) containing 10% (v/v) β-mercaptoethanol, passed through a syringe several times, and heated (65 °C, 5 min). Cell lysates were then centrifuged at 16249 g for 5 min, and ~30 μg protein were loaded onto a 10% SDS–PAGE gel and immunoblotted onto PVDF membranes. The membranes were blocked with 3% BSA blocking buffer (3% BSA in 1× wash buffer containing 50 mM Tris base, 150 mM NaCl, 1% Triton X-100, and 1% Nonidet P-40) for 1 h at room temperature and incubated overnight at 4 °C with primary antibodies (phosphorylated AMPK threonine-172, phosphorylated coflin serine-3 or β-actin (1:1000 dilution for each antibody)). The membranes were then washed and incubated with an appropriate HRP-coupled secondary antibody (1:5000 dilution) for 1 h. The membranes were subsequently washed, and proteins were visualized using ECL.

Statistical analysis

Data are expressed as means ± S.E.M. Statistical analysis was performed with GraphPad Prism 5 using paired Student’s t-test and one-way ANOVA followed by Tukey’s multiple-comparison test. Differences between the groups were considered statistically significant when P < 0.05.

Results

Actin cytoskeleton remodeling is an important component of insulin-stimulated GLUT4 translocation, and in this study, we observed a robust induction of actin and DAPI for nuclear staining. Each micrograph is a representative collapsed ‘xy projection’ image of n = 4 experiments taken under 60× oil immersion objective. Full colour version of this figure available via http://dx.doi.org/10.1530/JME-13-0059.
remodeling in membrane ruffles by gAd, which was reminiscent of the structures observed under insulin-treated (100 nM, 10 min) conditions (Fig. 1A and B). The remodeling induced by gAd occurred as early as 5 min (Fig. 1C) and was persistent after up to 30 min of treatment (Fig. 1D). Few cells displayed ruffling by 1 h of treatment (data not shown). gAd treatment induced both long lamellipodium membrane structures similar to those induced by insulin and distinct short ruffle structures on the dorsal surface of L6 myoblasts (bottom panel of Fig. 1C and D). The full-length form of adiponectin did not elicit the same degree of cytoskeletal remodeling (data not shown). When membrane morphology was examined with scanning electron microscopy, gAd was observed to increase the presence of membrane ruffles on the apical surface of L6 cells after 10 (Fig. 2D, see the arrows) and 30 (Fig. 2F, see the arrows) min of treatment, compared with the untreated cells, which have a smooth topography (Fig. 2A). Insulin was used as a positive control to induce membrane ruffles in these cells (Fig. 2B, arrows) and CD was used to disrupt the actin cytoskeleton network (Fig. 2C, E, and G). Data indicate that the effect of gAd on the formation of apical membrane ruffles was abolished when L6 cells were treated with CD and then with gAd for 10 (Fig. 2E) or 30 (Fig. 2G) min. We next examined whether gAd mediated the phosphorylation and inhibition of cofilin, an actin-severing protein. We observed increased phosphorylation of cofilin (S3) following 5–20 min of gAd treatment (Fig. 3).

We next established that gAd induced the translocation of LKB1 from the nucleus to the cytosol in L6 myoblasts (Fig. 4G, H, and I), as was observed previously in C2C12 myoblasts (Deepa et al. 2011). In untreated cells, the majority of LKB1 was localized to the nucleus (Fig. 4A, B, and C), and this was not markedly affected by CD treatment (Fig. 4D, E, and F) conditions. The ability of adiponectin to translocate LKB1 was disrupted when L6 cells were pretreated with CD (Fig. 4J, K, and L). The translocation of LKB1 to a cytosolic location in response to gAd (Fig. 5G) occurred in conjunction with actin remodeling (Fig. 5H) and was evidenced by the colocalization between cytosolic LKB1 and F-actin (Fig. 5I, see the arrows). This colocalization was abolished in cells treated with CD (Fig. 5J, K, and L).

**Figure 5**
Colocalization of LKB1 with gAd-induced actin membrane ruffles. The actin cytoskeleton network (rhodamine–phalloidin stain) and LKB1 localization (FITC) were visualized in L6-GLUT4myc myoblasts under (A, B, and C) control, (D, E, and F) CD-treated, (G, H, and I) gAd-treated (2.5 μg/ml for 30 min), and (J, K, and L) gAd+CD-treated conditions. Each micrograph was taken under 60× oil immersion objective and is a representative collapsed ‘xy projection’ image of n=3 experiments. Full colour version of this figure available via http://dx.doi.org/10.1530/JME-13-0059.
We also observed that the phosphorylation of AMPK after 10 and 20 min of gAd treatment was attenuated in the presence of CD (Fig. 6).

Actin remodeling and formation of membrane ruffles in response to insulin have been reported to be the key steps in insulin signaling leading to GLUT4 translocation and insertion into the plasma membrane, and we first confirmed the effects of insulin on actin remodeling and cell surface GLUT4 content here as positive controls (Fig. 7D, E and F). We did not observe any colocalization between cell surface GLUT4-myc and the actin structures induced by gAd (Fig. 7J, K, and L) or observe any effect of CD on the increased GLUT4-myc content on the cell surface (Fig. 7J, K, L, M, N, and O). Quantitative analysis of cell surface GLUT4-myc content using a colorimetric assay clearly demonstrated significant increases at 30 and 60 min of gAd treatment (Fig. 8A). CD did not have an effect on this translocation (Fig. 8A). However, we observed an inhibition of gAd-stimulated glucose uptake by CD (Fig. 8B). As expected, insulin-stimulated glucose uptake was also inhibited by CD (Fig. 8B).

**Discussion**

Adiponectin mediates potent anti-diabetic effects due to both insulin-mimetic and insulin-sensitizing actions, and potential therapeutic implications are evident (Yamauchi & Kadowaki 2008, Matsuzawa 2010, Dadson et al. 2011). To be able to manipulate the action of adiponectin, and ultimately prevent deficiencies in adiponectin function, it is imperative to further understand the cellular events underlying these effects. Adiponectin improves whole-body glucose homeostasis in part by stimulating skeletal muscle glucose uptake and metabolism (Ceddia et al. 2005, Satoh et al. 2005, Civitarese et al. 2006, Yoon et al. 2006, Vu et al. 2007, Liu et al. 2009). In this study, we examined the involvement of actin cytoskeleton remodeling in adiponectin-stimulated signaling and glucose uptake in skeletal muscle.

AMPK is a major regulator of many effects of adiponectin, including glucose uptake in the muscle. Indeed, the regulation of glucose transport by adiponectin in skeletal muscle is dependent on the interaction of APPL1 with AdipoR (Mao et al. 2006, Wang et al. 2007, 2009a, Zhou et al. 2009, Xin et al. 2011), leading to the subsequent activation of AMPK (Tomas et al. 2002, Yamauchi et al. 2002, Tsao et al. 2003, Ceddia et al. 2005, Fang et al. 2005, Mao et al. 2006, Wang et al. 2007, Zhou et al. 2009). Adiponectin-induced phosphorylation and activation of AMPK are primarily mediated through the cytosolic translocation of LKB1, which is mediated by PKC-ζ (Deepa et al. 2011), and the interaction of LKB1 with APPL1, which localizes it to the cytosol (Zhou et al. 2009, Fang et al. 2010). In this study, we also found that gAd enhances LKB1 content in the cytosol of L6 skeletal muscle cells. Our results demonstrate for the first time that actin remodeling is essential for this adiponectin-induced signaling as disassembly of the actin network with CD reduces LKB1 translocation to the cytosol and the degree of AMPK activation.

The novel observation that gAd treatment induced the remodeling of actin cytoskeleton in L6 myoblasts involved the formation of long lamellipodia membrane ruffles similar to those observed with insulin treatment (Khayat et al. 2000, Huang et al. 2005), and shorter small ruffles were also observed with gAd treatment. Since ruffles were observed on the dorsal surface of the cells, these structures are probably membrane protrusions (Ladwein & Rottner 2008), rather than focal adhesions (Xu et al. 2010), which are known to be locations for the fusion of GLUT4-containing vesicles (Tsakiridis et al. 1994, Khayat et al. 2000, Tong et al. 2001). A further difference between...
cytoskeletal reorganization in response to insulin and gAd was the temporal nature of these effects. Insulin has been shown to induce a transient remodeling, which was maximal at 10 min (Khayat et al. 2000, Huang et al. 2005), whereas actin remodeling following gAd treatment commenced at 5 min and was sustained for up to 30 min. To date, little is known regarding the functional significance of cytoskeletal remodeling by adiponectin.

The mixture of adipokines secreted by primary rat adipocytes, containing significant amounts of adiponectin, has been shown previously to increase glucose uptake in skeletal muscle cells in a CD-sensitive manner (Vu et al. 2011). Direct regulation of the actin cytoskeleton by adiponectin has been shown previously in primary adult cardiomyocytes to be important in the regulation of lipoprotein lipase movement and involved in the activation of RhoA and phosphorylation of cofilin (Ganguly et al. 2011).

Adiponectin has also been reported to induce migration activity in human endothelial progenitor cells through cytoskeleton remodeling regulated by the PI3K/Cdc42/Rac1 pathway (Nakamura et al. 2009). In human coronary artery endothelial cells, adiponectin suppresses cell migration induced by vascular endothelial growth factor through the inhibition of actin stress fiber formation (Mahadev et al. 2008). Thus, adiponectin induces specific remodeling of actin cytoskeleton in different cell types with diverse functional outcomes. Cofilin is an actin-severing protein known to regulate actin dynamics by depolymerizing F-actin, resulting in free actin monomers and creating barbed ends along F-actin structures, both of these functioning to maintain a steady state of actin polymerization (Oser & Condeelis 2009). In its unphosphorylated state, cofilin is active and dephosphorylates filamentous actin, resulting in free globular actin units (Ridley 2006, Oser & Condeelis 2009). When phosphorylated at serine-3 by LIMK1, cofilin is inactive and filamentous actin remains intact, thus allowing for the extension of the F-actin network (Bernard 2007, Oser & Condeelis 2009). We examined cofilin phosphorylation following gAd treatment and detected a rapid increase in cofilin phosphorylation on serine-3, suggesting that gAd enhances filamentous actin elongation through the deactivation of cofilin.

Glucose uptake in response to hormone-stimulated signaling involves the regulation of small GTPases leading to the redistribution of GLUT4 transporters to the plasma membrane followed by tethering and vesicle fusion with the cell membrane (Foley et al. 2011, Leto & Saltiel 2012).

Figure 7
Effect of gAd and CD on cell surface GLUT4 localization. Confocal fluorescence images of L6-GLUT4myc myoblasts stained with rhodamine–phalloidin for F-actin and FITC for GLUT4 were taken under (A, B, and C) control, (D, E, and F) insulin-stimulated (100 nM, 10 min), (G, H, and I) CD-treated, (J, K, and L) gAd-treated, and (M, N, and O) gAd + CD-treated conditions. Each image is a representative collapsed ‘xy projection’ image of n = 3 experiments taken using 60 x oil immersion objective. Full colour version of this figure available via http://dx.doi.org/10.1530/JME-13-0059.
The actin cytoskeleton has been shown to have an important role in the mediation of these events in response to insulin in skeletal muscle (Stockli et al. 2011). We confirmed these observations in the current study and observed that whereas the activation of AMPK and stimulation of glucose uptake involved cytoskeletal remodeling, GLUT4 detection at the cell surface in response to adiponectin occurred independent of this process. This distinction between GLUT4 translocation and glucose uptake has been observed previously (Furtado et al. 2002). Our data suggest that the regulation of glucose transport into skeletal muscle cells but not translocation of GLUT4 depends upon adiponectin-stimulated cytoskeleton remodeling.

In summary, we add new insights into the mechanisms underlying the metabolic action of adiponectin in skeletal muscle by demonstrating a requirement for cytoskeletal remodeling in LKB1 translocation and activation of AMPK leading to glucose uptake. These findings also indicate similarities and divergent aspects of the regulation of skeletal muscle glucose uptake by insulin and adiponectin.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Author contribution statement**

V V was responsible for the experimental design, majority of experiments, data analysis, and manuscript writing; P B and M E conducted the experiments; A X was responsible for the planning of the experimental approach, supply materials and reagents, and manuscript revision; and G S was responsible for the experimental concept and design, data analysis, manuscript writing, and funding.

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**References**


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**Figure 8**

Effect of gAd and cytoskeleton disruption on GLUT4 translocation and glucose uptake in L6-GLUT4<sup>myc</sup> myoblasts. (A) GLUT4 localization to the plasma membrane and (B) uptake of 2-deoxyglucose were measured in L6-GLUT4<sup>myc</sup> myoblasts following treatment with 2.5 μg/ml gAd. Data are presented as fold increase above the control in which control (Con; without CD) was set to 1 and insulin (Ins) was used as a positive control, and data presented as fold increase above the control in which control (Con; without CD) and *P<0.05 vs the respective insulin (Ins), adipocyte-conditioned media, or CD treatment without CD.

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**Author contribution statement**

V V was responsible for the experimental design, majority of experiments, data analysis, and manuscript writing; P B and M E conducted the experiments; A X was responsible for the planning of the experimental approach, supply materials and reagents, and manuscript revision; and G S was responsible for the experimental concept and design, data analysis, manuscript writing, and funding.

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