An APPL1-AMPK signaling axis mediates beneficial metabolic effects of adiponectin in the heart

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Fang X, Palanivel R, Cresser J, Schram K, Ganguly R, Thong FS, Tuinei J, Xu A, Abel ED, Sweeney G. An APPL1-AMPK signaling axis mediates beneficial metabolic effects of adiponectin in the heart. Am J Physiol Endocrinol Metab 299: E721–E729, 2010. First published August 24, 2010; doi:10.1152/ajpendo.00086.2010.—Adiponectin promotes cardioprotection via various mechanisms, and this study used primary cardiomyocytes and the isolated working perfused heart to investigate cardiometabolic effects. We show in adult cardiomyocytes that adiponectin increased CD36 translocation and fatty acid uptake as well as insulin-stimulated glucose transport and Akt phosphorylation. Immunoprecipitation showed that adiponectin enhanced association of AdipoR1 with APPL1, subsequent binding of APPL1 with AMPKα2, which led to phosphorylation and inhibition of ACC and increased fatty acid oxidation. Using siRNA to effectively knockdown APPL1 in neonatal cardiomyocytes, we demonstrated an essential role for APPL1 in mediating increased fatty acid uptake and oxidation by adiponectin. Importantly, enhanced fatty acid oxidation in conjunction with AMPK and ACC phosphorylation was also observed in the isolated working heart. Despite increasing fatty acid oxidation and myocardial oxygen consumption, adiponectin increased hydraulic work and maintained cardiac efficiency. In summary, the present study documents several beneficial metabolic effects mediated by adiponectin in the heart and provides novel insight into the mechanisms behind these effects, in particular the importance of APPL1.

AMP-activated protein kinase; fatty acid; metabolism

There is currently great interest in elucidating the mechanisms by which obesity can influence myocardial remodeling (2). Changes in myocardial energy metabolism are one of the earliest measurable abnormalities in the hearts of obese animals or humans and precede measurable changes in in vivo cardiac function (1, 7, 16, 31, 32, 41). Shifts in myocardial substrate utilization in obesity and diabetes are typically characterized by an increase in fatty acids (FA) utilization and a decrease in glucose utilization (34). Multiple mechanisms account for these changes in metabolism and include altered glucose transport (42), increased delivery of FA, and activation of PPARα-mediated signaling pathways (2). A well controlled balance of FA uptake and oxidation is essential in maintaining both ATP production and cardiac contractile function and may also prevent potential adverse effects associated with lipotoxicity. For example, elevated FA uptake that is not matched by a proportionate increase in FA oxidation may contribute to the accumulation of intracellular triglycerides and lipotoxic products such as ceramide, diacylglycerol, and fatty acyl-CoA, which have widespread detrimental cellular consequences (38).

Obese models such as Zucker rats exhibit a decreased ability to increase FA oxidative capacity in response to increasing FA delivery, and this has been suggested to contribute to accumulation of myocardial triglycerides and lipotoxicity (35, 46). Although, ob/ob and db/db mice have increased capacity to oxidize FA in response to increasing delivery of FA substrates, which exceeds that of wild-type hearts, these animals also exhibit evidence of lipid accumulation and lipotoxicity, mitochondrial uncoupling, and decreased cardiac efficiency (6, 7, 26). Recent studies in obese humans have yielded results that mirror the changes described in mice. In a study of severely obese females, obesity was associated with increased rates of FA oxidation, increased myocardial oxygen consumption (MVΩO2), and reduced cardiac efficiency (32).

Adiponectin has now been extensively documented to mediate several cardioprotective effects (2, 28), many of which appear to be mediated via AMPK (21, 36, 37). Although the role of adiponectin in modulating carbohydrate and lipid metabolism has been extensively studied in muscle and liver (4, 15, 39, 44, 45), only a few studies to date have investigated direct metabolic effects of adiponectin on cardiomyocyte metabolism (13, 21, 27, 29, 33). Because obesity and insulin resistance are associated with hypoadiponectinemia, the present study tested the hypothesis that adiponectin exerts direct effects on cardiomyocyte FA metabolism, which could potentially influence cardiac contractile function. Two adiponectin receptor isoforms, AdipoR1 and AdipoR2, have been characterized (43), which are now known to interact with APPL1 (adaptor protein-containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif) to mediate downstream signaling (10, 25). In this study, we characterized the effect of adiponectin on FA uptake and metabolism in cardiomyocytes and perfused hearts and investigated the mechanistic role of the APPL1-AMPK axis.

Materials. 2-Deoxy-D-[3H]glucose was purchased from Amersham (Quebec, Canada). The AMPK inhibitor compound C was purchased from Calbiochem (San Diego, CA). Insulin (Humulin) was obtained from Eli Lilly (Toronto, ON, Canada). Horseradish peroxidase (HRP)-linked anti-rabbit antibody, β-actin antibody, acetyl-CoA carboxylase (ACC) antibody, phosphospecific antibodies for rabbit AMPK (Thr172), ACC (Ser79), and Akt (Thr308) were purchased from Cell Signaling (Beverly, MA). AMPKα1, AMPKα2, and LKB1 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). AdipoR1 and AdipoR2 antibodies were obtained from IBL (Takasaki, Japan). Full-length FLAG-tagged adiponectin, which contained an
oligomeric profile similar to that found in circulation, was produced in a mammalian expression system (HEK 293 cells). We used both adiponectin-containing conditioned media and adiponectin purified, using an anti-FLAG M2 monoclonal antibody affinity column (29). Anti-APPL1 antibody was produced by immunization of rabbits as previously described (10). Oil red O and triethyl phosphate were purchased from Fluka Chemie (Buchs, Switzerland). All other chemicals were purchased from Sigma (St. Louis, MO), and cell culture components were purchased from Wisent (Quebec, Canada).

Isolation of adult and neonatal cardiomyocytes. Adult rat cardiomyocytes were isolated from male Wistar rats (250–350g). Rats were anesthetized with ketamine (100 mg/kg ip) and hearts were rapidly excised and retrogradely perfused with Krebs-Henseleit solution (in mM): 4.8 mM KCl, 1.25 mM K$_2$HPO$_4$, 118 mM NaCl, 1.25 mM MgSO$_4$, 25 mM HEPES, 10 mM glucose, 10 mM butanedione, pH 7.4, and l-berose (0.18 mg/ml, Roche Applied Science) at 37°C. The heart was then minced, and cardiomyocytes were isolated by sedimentation in gradually increasing calcium concentration until a final concentration of 1 mM was achieved. The cardiomyocytes were gently resuspended in serum-free Medium 199 and plated on laminin (10 μg/ml, Sigma)–precoated plates. After an adhesion period of 2 h, cardiomyocytes were cultured in Medium 199 supplemented with 5% FBS and antibiotics (100 IU/ml penicillin + 10 mg/ml streptomycin, Multicell). Primary cultures of neonatal cardiomyocytes were isolated from the ventricles of 2- to 3-day-old Wistar rats by enzymatic digestion as described previously (29). The care and use of animals for these experiments were approved by the York University Animal Care Committee (Toronto, ON, Canada).

Measurement of metabolism in vitro and in isolated working mouse hearts. FA uptake and oxidation were measured as previously described (14, 45). ACC activity was assayed by measuring the incorporation of [14C]sodium bicarbonate into malonyl-CoA (29). Substrate metabolism was determined in hearts that were isolated from 6-wk-old C57BL6 mice (Jackson Laboratory, Bar Harbor, ME) by use of previously published protocols (26). The Krebs-Henseleit buffer was supplemented with 0.4 mM palmitate bound to 3% BSA and 5 mM glucose with or without adiponectin (4 μg/ml, final concentration). Hearts were perfused for 60 min, and rates of glycolysis, glucose oxidation and palmitate oxidation, MV˙O$_2$, hydraulic work, and cardiac efficiency were determined as previously described (26). These studies were approved by the Institutional Animal Care and Use Committee of the University of Utah.

Immunoblotting, immunohistochemistry and immunoprecipitation. Sarcolemmal abundance of CD36 was determined by immunofluorescence using a specific anti-CD36 polyclonal antibody that recognizes the extracellular domain of CD36 essentially as previously described (30). Briefly, myocytes were cultured on coverslips and incubated with adiponectin (10 μg/ml). Myocytes were then quickly washed with PBS and incubated with anti-CD36 (H300; Santa Cruz Biotechnology, 1:200 dilution) for 60 min at 4°C. After myocytes were fixed in 3% paraformaldehyde for 3 min on ice, the fixative was neutralized by incubation in 1% glycine for 10 min. Myocytes were incubated in blocking buffer (5% goat serum + 3% BSA) for 30 min and then were incubated with Alexa fluor 488-conjugated secondary antibody (Molecular Probes, Invitrogen, 1:1000 dilution, 4°C) for 30 min. The images (stacks of optical slices) of CD36 translocation were quantified by image J software. Cell surface CD36 content in neonatal cardiomyocytes was determined by an antibody-coupled calorimetric assay (14). Briefly, cells were grown in 24-well plates and, after treatment with adiponectin (10 μg/ml), washed with ice-cold PBS and incubated with anti-CD36 antibody (1:200 dilution) for 60 min at 4°C. Myocytes were fixed in 3% paraformaldehyde for 3 min on ice, and the fixative was neutralized by incubation in 1% glycine for 10 min. Cells were then incubated in blocking buffer (5% goat serum + 3% BSA) for 30 min and incubated with HRP-conjugated goat-anti-rabbit antibody (1:1000 dilution) for 60 min at 4°C. After a wash with ice-cold PBS, myocytes were incubated for 30 min at room temperature with 1 ml of o-phenylenediamine dihydrochloride (OPD) reagent (Sigma) per well. The reaction was stopped by adding 0.25 ml of HCl (3M), supernatant was collected, and absorbance was measured at 492 nm. To detect the translocation of APPL1, myocytes were double-immunostained with a mAb against dystrophin (Abcam) and an anti-APPL1 antibody. Representative images (optical single slice) of APPL1 translocation was selected to show the distribution of APPL1 close to the plasma membrane (indicated by dystrophin staining). Nuclear and cytosol fractions were prepared using a nuclear/cytosol fractionation kit (BioVision, Mountain View, CA), and the level of LKB1 was then determined by Western blotting. Colocalization of LKB1 and APPL1 in primary cardiomyocytes were determined by immunoprecipitation. To prepare homogenates for Western analyses, perfused hearts were flash frozen, pulverized under liquid nitrogen and then processed as described previously (5), with slight modifications.

Determination of intracellular lipid content. To examine the effect of adiponectin on lipid content, adult cardiomyocytes were grown on coverslips in six-well culture plates and incubated with or without adiponectin (10 ng/ml) for 24 h, and then lipid content was measured using methods as previously described (30). Briefly, cardiomyocytes were fixed with 3.7% formaldehyde and stained with Oil red O. Imaging was performed on a laser scanning confocal microscope (Olympus fluorescence 300). The intensity of intracellular lipid content was then analyzed with Image J software.

Measurement of FA uptake and oxidation and ACC activity. FA uptake was measured using fluorescent palmitate as previously described (14). ACC activity was assayed in isolated adult cardiomyocytes by measuring the incorporation of [14C]sodium bicarbonate into malonyl-CoA (29).

siRNA-mediated knockdown of APPL1 in neonatal rat primary cardiomyocytes. Several 21-nucleotide small interfering RNA (siRNA) sequences (Ambion, Austin, TX) designed to knock down rat APPL1 were tested in cardiomyocytes, which were incubated for 1 h in serum-free medium prior to transfection with siRNAs. The sequence that provided optimal efficiency was: APPL1, 5'-CUUAGAUCUUGU-GAUCGCAU-3' (50 nM was transfected into myocytes using the TransIT-TKO reagent (MirusBio, Madison, WI). After 24 h, the medium was replaced by serum-free medium, and cells were then treated with adiponectin (30 nM) as previously described. After 24 h of siRNA transfection, cells were washed with PBS and lysed with 300 μl of cell lysis buffer [0.5 M Tris-HCl (pH 6.8), 10% (vol/vol) SDS, 15% (vol/vol) glycerol, 10% (vol/vol) β-mercaptoethanol, 0.2 mM PMSF, 10 μg/ml leupeptin, 1 mM pepstatin A, 0.5 mM Na$_3$VO$_4$, 0.2 mM E64, and bromophenol blue]. The lysates were then boiled at 65°C for 5 min, then syringed 5 times, and centrifuged at 12,000 rpm for 1 min at 4°C. Equal amounts of lysate were resolved in 8% of SDS-PAGE and immediately transferred to PVDF membranes, which were then probed overnight at 4°C with APPL1 antibody. Bands were visualized by chemiluminescence after a 1-h incubation with HRP-conjugated secondary antibody and also probed with β-actin to control for loading. For cells with APPL1 knockdown, FA uptake and oxidation, in the presence or absence of adiponectin (1 h) were determined using [3H]palmitate and [1-14C]palmitate, as we previously described (29).

Measurement of glucose uptake and insulin signaling. Glucose uptake was determined by measuring uptake of 2-deoxy-D-[3H]glucose as described previously (14). Briefly, adult cardiomyocytes were pretreated with adiponectin (10 μg/ml) for 15 min followed by insulin (10 nM) treatment for 5 min in the continued presence of adiponectin. After treatment, cardiomyocytes were incubated in transport solution (140 mM NaCl, 20 mM HEPES-Na, 2.5 mM MgSO$_4$, 1 mM CaCl$_2$, 5 mM KCl, 10 μM 2-deoxyglucose, 0.5 μM/ml 2-deoxy-[3H]glucose, pH 7.4) for 5 min at room temperature. Cells were then lysed and transferred to scintillation vials for [3H]radioactivity counting. Values were calculated as picomoles per milligram of protein per minute. Phosphorylation of Akt at Thr$^{308}$ and total Akt level were determined.
by Western blot. Cardiomyocytes were incubated with insulin (10 nM) for 5 min with adiponectin (10 μg/ml) pretreatment for 15 min, and then lysates were prepared and analyzed as described before (14).

**Statistical analysis.** Data are expressed as mean values ± SE and number of replicates (n) stated in each case. Statistical analysis was undertaken using one-way ANOVA with Student-Newman-Keuls post hoc analysis or the paired Student’s t-test where appropriate. Differences between groups were considered statistically significant when P < 0.05.

**RESULTS**

Adiponectin increases CD36 translocation and FA uptake and insulin sensitivity in primary adult cardiomyocytes. The ability of physiologically relevant levels of adiponectin (10 μg/ml) to induce long-chain FA uptake in adult rat cardiomyocytes was assessed with fluorescence-labeled palmitate. Palmitate uptake was increased following 30 and 60 min of adiponectin treatment (Fig. 1A). Insulin served as a positive control. Although similar observations were made previously in neonatal cardiomyocytes, the mechanism remained unclear. Therefore, we measured CD36 translocation and membrane insertion in intact cells with an antibody directed against an exofacial epitope of CD36. Representative fluorescent images clearly show enhanced cell surface CD36 in response to adiponectin in primary adult cardiomyocytes, and quantitative analysis of multiple experiments showed increases of 1.5-fold at 15 and 30 min, respectively (Fig. 1B). Adiponectin also increased basal and insulin stimulated glucose uptake, and increased the ability of insulin to phosphorylate Akt. (Fig. 1, C and D).

**APPL1-dependent signaling plays an essential role in regulating fatty acid metabolism in response to adiponectin.** We next examined whether adiponectin increased the association of APPL1 with either of two adiponectin receptor (AdipoR) isoforms in adult cardiomyocytes. Figure 2A shows coimmunoprecipitation evidence of binding of APPL1 to both receptor isoforms under basal conditions and increased binding of APPL1 to AdipoR1 but not AdipoR2 in response to adiponectin. We also examined intracellular localization of APPL1 in...
Fig. 2. Functional participation of APPL1 in Ad’s physiological actions in isolated rat cardiomyocytes. Representative immunoblots (IB) showing interaction between APPL1 and adiponectin receptor 1 (AdipoR1) or AdipoR2 (A) and AMPKα1 or AMPKα2 catalytic subunits (C). B: representative confocal images (optical single slice) showing individual and merged staining of cell surface APPL1 (green) or sarcolemma marker dystrophin (red). Enlarged segment is an optical single slice, magnification ×60. D: immunoblot showing knockdown of endogenous APPL1 (si-APPL1) compared with nontransfected (Control) neonatal rat cardiomyocytes. Palmitate uptake (E), palmitate oxidation (F), and CD36 translocation (G) in isolated neonatal rat cardiomyocytes not transfected (Control) or transfected with an unrelated siRNA (SCR) or siRNA against APPL1 (si-APPL1) and were treated with or without 10 μg Ad for 1 h (E and F) or 30 min (G). H: representative immunoblot showing AMPK phosphorylation (Thr172) in isolated neonatal rat cardiomyocytes transfected with SCR or si-APPL1 and subsequently treated with or without 10 μg Ad for 10 min. Data are means ± SE of ≥4 independent experiments and are expressed relative to Control (0 min). *P < 0.05 vs. Control (0 min).
adult rat primary cardiomyocytes by immunofluorescence and observed that adiponectin increased colocalization of APPL1 with the sarcolemmal marker dystrophin and increased subplasma membrane localization (Fig. 2B). Adiponectin also selectively increased the interaction between APPL1 and AMPKα2 but not with AMPKα1 subunit isoform (Fig. 2C). To elucidate the functional significance of APPL1-dependent adiponectin signaling, we utilized siRNA to reduce APPL1 expression in primary neonatal cardiomyocytes to ~30% of endogenous levels (Fig. 2D), as gene silencing was not feasible in primary adult cardiomyocytes. Subsequent analysis of FA uptake (Fig. 2E), FA oxidation (Fig. 2F), and CD36 translocation (Fig. 2G) showed that APPL1 siRNA, but not scrambled siRNA, significantly attenuated the ability of adiponectin to increase FA uptake and oxidation. Importantly, adiponectin-induced stimulation of AMPK phosphorylation was also blunted by the siRNA-mediated reduction of APPL1 level in neonatal cardiomyocytes (Fig. 2H).

**Adiponectin stimulates LKB1/AMPK signaling to enhance FA uptake and oxidation.** An important regulatory step in activation of AMPK by adiponectin in skeletal muscle is translocation of LKB1, an AMPK kinase, from nucleus to cytosol. Thus, we investigated the interaction between APPL1 and AMPK signaling, which has been proposed to mediate many of the metabolic effects of adiponectin. In adult cardiomyocytes, adiponectin decreased nuclear and increased cytosolic LKB1 content (Fig. 3A). Importantly, we also showed via coimmunoprecipitation that this correlated with enhanced association of LKB1 with APPL1 in response to adiponectin (Fig. 3B) and subsequent phosphorylation of AMPK (Fig. 3C). Functional consequences of this signaling axis were again analyzed, and we observed that inhibition of AMPK in adult cardiomyocytes with compound C significantly attenuated adiponectin-stimulated FA uptake (Fig. 3D). Adiponectin signaling also increased the phosphorylation of ACC (Fig. 4A), thereby inhibiting ACC activity (Fig. 4B). We ultimately confirmed the ability of adiponectin to regulate FA oxidation and demonstrated increased palmitate oxidation after 2 h (Fig. 4C).

Long-term treatment of adult cardiomyocytes with adiponectin led to a decrease in cardiomyocyte lipid content determined by Oil red O staining as shown by representative image (Fig. 4D) and by quantitative analysis (Fig. 4E).

**Adiponectin enhances AMPK/ACC signaling, FA metabolism, and cardiac power in isolated working hearts.** In isolated working hearts, adiponectin, immunoblot analysis of whole heart homogenates obtained from perfused hearts revealed a significant increase in the phosphorylation of AMPK and ACC (Fig. 5, A and B). Expression levels of AMPKα subunits, LKB1, APPL1, and ACC are shown and were unaltered after adiponectin perfusion (Fig. 5C). Under these conditions, adiponectin significantly increased palmitate oxidation (67%) (Fig. 5D), whereas glucose oxidation (Fig. 5E), and glycolysis (Fig. 5F) were both decreased by ~30%. MV˙O2 (Fig. 5G) and

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**Fig. 3. Ad activates LKB1-AMPK signaling to increase FA metabolism in adult primary rat cardiomyocytes.**

A: representative immunoblot and quantification of LKB1 in cytosolic and nuclear fractions in cells untreated or treated with 10 μg Ad for 5 min. B: representative immunoblot showing interaction between LKB1 and APPL1. C: representative immunoblot and quantification of AMPK phosphorylation (Thr172). D: quantification of palmitate uptake in unstimulated (Control) or stimulated with 10 μg Ad for indicated times with or without 30-min pretreatment with 10 μM compound C. Data are means ± SE of ≥4 independent experiments and are expressed relative to Control (0 min or without adiponectin). *P < 0.05 vs. Control.

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**Fig. 4. Adiponectin does not change ACC expression but increases ACC phosphorylation, FA oxidation, and reduces lipid content in adult cardiomyocytes.** Data are means ± SE of ≥4 independent experiments. *P < 0.05 vs. Control.
DISCUSSION

Obesity is an established risk factor for heart failure, and many studies have strived to unravel the mechanisms responsible for the cardiac remodeling characteristic of obesity (2). Accordingly, adipokines have emerged as potentially important components of the pathophysiology of heart failure. In this study, we focused on adiponectin, which has been shown to exert multiple cardioprotective actions, including antiapoptotic, antifibrotic, and antihypertrophic effects (28). Because altered cardiac metabolism is one of the earliest detectable remodeling events in the progression toward heart failure (2), we previously examined the direct effects of adiponectin on palmitate and glucose metabolism in neonatal rat cardiomyocytes (29). We now provide more detailed analyses of mechanisms underlying adiponectin’s cardiometabolic effect in primary adult cardiomyocytes and isolated working mouse hearts.

Adult cardiomyocytes are more reliant on FA than neonatal cardiomyocytes and respond to adiponectin by increasing palmitate uptake and oxidation. An important role for translocation of the long-chain FA transporter CD36 in cardiac metabolism has been suggested in response to stimuli such as contraction and insulin (9, 23, 24). Our results indicate that adiponectin-stimulated FA uptake in adult rat cardiomyocytes correlates with mobilization of CD36 and was a consequence of AMPK activation. Adiponectin also regulates the metabolic fate of FAs. ACC plays an important role in cardiac FA oxidation (3, 17). We observed that adiponectin increased ACC phosphorylation, with a corresponding decrease in activity, and a sustained increase in FA oxidation. Therefore, adiponectin to a certain extent mimics the impact of exercise on myocardial FA utilization. We also demonstrate in adult cardiomyocytes that adiponectin can increase basal and insulin-stimulated glucose uptake and enhance insulin-stimulated Akt phosphorylation.

Observations made regarding the regulation of metabolism by adiponectin in primary adult cardiomyocytes were supported by studies in isolated working hearts, wherein adiponectin increased rates of myocardial FA oxidation and V\textsubscript{O\textsubscript{2}}. Because there was a proportionate increase in cardiac function, there was no reduction in cardiac efficiency. This contrasts with observations in animal models of obesity and diabetes in which FA oxidation and M\textsubscript{V\textsubscript{O\textsubscript{2}}} are increased but cardiac function and cardiac efficiency are reduced (7, 26). The reduction in cardiac efficiency in obesity is due in part to mitochondrial uncoupling and the futile FA cycling secondary to increased expression and activity of mitochondrial and cytosolic thioesterases (8). Thus, the present study suggests that adiponectin may mediate cardioprotection by increasing the efficient oxidation of FAs. It is likely that this mechanism is mediated in part by activation of AMPK. AMPK also increases GLUT4 translocation and glycolysis (20). However, the sig-
significant increase in FA oxidation in these aerobic hearts leads to reciprocal reduction in glucose utilization via the Randle Cycle. We propose that, in contrast to the changes observed in diabetes, adiponectin promotes a shift in FA utilization that is adaptive. In the heart, lipotoxicity is associated with depressed contractile function, cardiac hypertrophy, and cardiomyocyte apoptosis (11, 12, 35, 48). Increased mitochondrial FA oxidation (as occurs in response to AMPK activation) could potentially prevent or reverse cardiac lipid accumulation. Our data therefore raise the intriguing possibility that adiponectin may play a protective role in limiting lipid accumulation by enhancing cardiac FA oxidation while maintaining normal cardiac efficiency.

We have shown previously (19) that adiponectin mediates many of its effects, including cardiomyocyte metabolism (29), through receptors AdipoR1 and AdipoR2. Until recently, the immediate downstream effector of AdipoR-mediated signaling was unknown, but studies have now identified APPL1 as a novel component of adiponectin signaling (10, 25, 40). Studies by Xu’s and Dong’s laboratories (10, 25) showed that APPL1 plays a vital role in adiponectin-stimulated NO production and glucose transport in endothelial cells and C2C12 skeletal muscle cells, respectively. Here, we show for the first time in primary adult rat cardiomyocytes that APPL1 regulates adiponectin signaling and FA metabolism. Adiponectin stimulated the apparent recruitment of APPL1 to a sub-plasma membrane localization, which is in keeping with binding to AdipoRs located in the plasma membrane. Indeed, our immunoprecipitation studies revealed that APPL1 interacted directly with AdipoR1 and AdipoR2 and specifically identified that adi-
Adiponectin increased the interaction between APPL1 and AdipoR1, but not AdipoR2, in this cell type. A similar interaction was also found in C2C12 cells in response to adiponectin (25). To determine the functional significance of APPL1 regulation by adiponectin in stimulating FA uptake and oxidation, we used neonatal cardiac myocytes in which APPL1 expression was suppressed by siRNA. Adiponectin-stimulated FA uptake and oxidation were significantly reduced in the APPL1-suppressed neonatal cardiomyocytes compared with the scrambled siRNA-treated control cells. Adiponectin-mediated CD36 translocation was also attenuated in cardiomyocytes where APPL1 had been knocked down. These results indicate that adiponectin regulates FA uptake and metabolism via an APPL1-dependent signaling mechanism.

AMPK plays a central role in mediating many of the physiological effects of adiponectin (18, 19). AMPK is regulated by AMP and phosphorylation on Thr172. We (29) previously reported that adiponectin mediates metabolic effects in neonatal rat primary cardiomyocytes by activating AMPK. We now show that the activation of AMPK by adiponectin in adult cardiomyocytes is mediated by recruitment of LKB1 to a complex that contains AdipoR1, APPL1, and AMPKα2. LKB1 is thought to be the major upstream kinase, which activates AMPK on Thr172 (22). It has been proposed that the binding of APPL1 to AdipoR is associated with translocation of LKB1 from nucleus to cytosol, interaction with APPL1, and phosphorylation of AMPK (47). Here, we observed that under basal conditions LKB1 was located predominantly in the nucleus of adult rat primary cardiomyocytes, and the translocation of LKB1 to the cytosol and association with APPL1 were also significantly increased by adiponectin. Although we did not investigate whether adiponectin altered LKB1 binding to STRADα or MO25, Zhou et al. (47) did not observe any such changes in C2C12 cells. Taken together, our findings support the existence of a signaling axis in cardiomyocytes involving APPL1, LKB1, and AMPKα2, which mediates the increase in FA uptake and oxidation in response to adiponectin.

The effects of adiponectin on cardiac metabolism and function are presently of great interest, and our current study in both primary cardiomyocytes and isolated working hearts suggests a beneficial role for adiponectin in the regulation of cardiac metabolism. We demonstrate that adiponectin increased cardiac function by promoting efficient utilization of fatty acid, and we speculate that this effect could potentially have therapeutic utility in limiting or reversing lipotoxicity in cardiac myocytes. We also provide important mechanistic insights by identifying APPL1 as an important mediator of adiponectin’s direct metabolic effects.

**REFERENCES**


20. Li J, Hu X, Selvakumar P, Russell RR, 3rd Cushman SW, Holman GD, Young LH. Role of the nitric oxide pathway in AMPK-mediated...


