

Metabolically Favorable Remodeling of Human Adipose Tissue by Human Adenovirus Type 36

Pamela M. Rogers,¹ Nazar Mashtalir,¹ Miloni A. Rathod,² Olga Dubuisson,¹ Zhong Wang,¹ Kumar Dasuri,¹ Scott Babin,¹ Alok Gupta,¹ Nathan Markward,¹ William T. Cefalu,¹ and Nikhil V. Dhurandhar¹

OBJECTIVE—Experimental infection of rats with human adenovirus type 36 (Ad-36) promotes adipogenesis and improves insulin sensitivity in a manner reminiscent of the pharmacologic effect of thiazolidinediones. To exploit the potential of the viral proteins as a therapeutic target for treating insulin resistance, this study investigated the ability of Ad-36 to induce metabolically favorable changes in human adipose tissue.

RESEARCH DESIGN AND METHODS—We determined whether Ad-36 increases glucose uptake in human adipose tissue explants. Cell-signaling pathways targeted by Ad-36 to increase glucose uptake were determined in the explants and human adipose-derived stem cells. Ad-2, a nonadipogenic human adenovirus, was used as a negative control. As a proof of concept, nondiabetic and diabetic subjects were screened for the presence of Ad-36 antibodies to ascertain if natural Ad-36 infection predicted improved glycemic control.

RESULTS—Ad-36 increased glucose uptake by adipose tissue explants obtained from nondiabetic and diabetic subjects. Without insulin stimulation, Ad-36 upregulated expressions of several proadipogenic genes, adiponectin, and fatty acid synthase and reduced the expression of inflammatory cytokine macrophage chemoattractant protein-1 in a phosphatidylinositol 3-kinase (PI3K)-dependent manner. In turn, the activation of PI3K by Ad-36 was independent of insulin receptor signaling but dependent on Ras signaling recruited by Ad-36. Ad-2 was nonadipogenic and did not increase glucose uptake. Natural Ad-36 infection in nondiabetic and diabetic subjects was associated with significantly lower fasting glucose levels and A1C, respectively.

CONCLUSIONS—Ad-36 proteins may provide novel therapeutic targets that remodel human adipose tissue to a more metabolically favorable profile. *Diabetes* 57:2321–2331, 2008

Obesity is associated with adverse metabolic profile of adipose tissue, including impaired adipogenesis, lower fatty acid synthase (FAS) and adiponectin, and increased secretion of inflammatory cytokines. Consequentially, this contributes to an increase in insulin resistance and a reduction in glucose uptake by the tissue (1–4). Although intentional

weight loss could improve insulin resistance and attenuate the adverse metabolic profile, achieving meaningful fat loss and maintaining it long term is very challenging. Instead, a particularly appealing approach proposes to “remodel” the adipose tissue to a more favorable or healthy metabolic profile. For instance, the thiazolidinedione (TZD) class of drugs increases glucose uptake in response to insulin stimulation (5), induces peroxisome proliferator-activated receptor (PPAR) γ 2, increases adipogenesis (6), activates phosphatidylinositol 3-kinase (PI3K) (7), reduces the release of inflammatory cytokines (8,9), and upregulates adiponectin secretion (10) and FAS expression (4) in adipose tissue. The metabolically beneficial effects of the TZDs and other remedial candidates, such as benzopyran-derived T33 (11), suggest that adipose tissue remodeling may be a pragmatic approach against the growing epidemic of diabetes. Among the other effects, expansion of adipose tissue by the TZDs appears to offer “storage space” for lipids (5,12) and offset ectopic lipid accumulation in muscle and liver, thereby contributing to insulin sensitivity.

Recently, there is considerable interest in the role of adipose tissue expansion in improving insulin sensitivity. Medina-Gomez et al. (13) showed that PPAR γ 2 controls adipose tissue expansion and thereby improves insulin sensitivity in *ob/ob* mice. Kim et al. (14) achieved dramatic improvement in metabolic profile through expansion of adipose tissue in transgenic mice. Despite the massive increase in adiposity, the improved metabolic profile comprised of greater glucose disposal and adiponectin secretion; reduction in serum cholesterol, triglycerides, and inflammation; and induction of expression of PPAR γ 2 and its target genes (14).

Human adenovirus type 36 (Ad-36) is another novel candidate for improving metabolic profile by expanding adipose tissue. Although Ad-36 increases adiposity (15–17), it enhances insulin sensitivity in experimentally infected rats (18) and reduces serum cholesterol and triglycerides (15–17). Indeed, a single inoculation of Ad-36 increased fat depot weight of rats by >60% but reduced the fasting insulin levels and homeostasis model assessment index by ~50% for up to 7 months later (18), a robust and long-term effect that is reminiscent of TZDs. Moreover, Ad-36 upregulates PPAR γ 2 expression and induces differentiation and lipogenesis in human and rodent preadipocytes (19–23) and increases glucose uptake in rat adipocytes (22), even in the absence of insulin, which possibly contributes to its insulin-sensitizing effect.

Harnessing certain properties of viruses for beneficial purposes has been creatively used for several years. For instance, even before the advent of antibiotics, the use of bacteriocidal properties of bacteriophage virus has been reported and had a recent resurgence in interest (rev. in

From the ¹Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana; and the ²Department of Nutrition, Wayne State University, Detroit, Michigan.

Corresponding author: Nikhil V. Dhurandhar, nikhil.dhurandhar@pbrc.edu.

Received 13 September 2007 and accepted 11 June 2008.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 3 July 2008.

DOI: 10.2337/db07-1311.

© 2008 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

24). Furthermore, the first report of the oncolytic ability of a mutant adenovirus (25) was followed by numerous studies extending the use of oncolytic properties of various viruses. Herpes simplex virus, Newcastle disease virus, reovirus, and vaccinia virus were used for the treatment of various cancers (rev. in 26), alone or in combination with various synergistic drugs (27,28). The potential of Ad-36 proteins to enhance glucose disposal and improve adipose tissue metabolic profile could be exploited as therapeutic targets for humans, a natural host of the virus. To investigate this possibility, we first determined whether Ad-36 increases glucose uptake in primary adipose tissue explants obtained from healthy and diabetic subjects. Next, we elucidated the cellular signaling pathways involved in an Ad-36-induced increase in glucose uptake. Finally, as a proof of concept, nondiabetic and diabetic subjects were screened for the presence of Ad-36 antibodies to ascertain if natural infection with Ad-36 was a significant predictor of improved glycemic control.

RESEARCH DESIGN AND METHODS

Human adipose tissue samples were obtained from nine healthy, nondiabetic female patients and one type 2 diabetic female patient undergoing elective liposuction surgeries. The samples were received as material to be discarded and without identifiers. Therefore, approval of the human investigations committee was not required for the study. The donors were Caucasian, were aged 28–50 years (mean 39 ± 9.5), and had BMI 19.5–28.2 kg/m² (mean 23.7 ± 3.3). Small chunks of adipose tissue were cultured as explants, as described below, and used to elucidate the effect of Ad-36 infection. Human adipose-derived stem cells (hASCs) were obtained from subjects with BMI 25–30 kg/m², aged 47–58 years. At least three or more technical replicates were used for all experiments. Samples from two or more subjects were used for Ad-36 experiments, except for the diabetic sample. The effect of Ad-2 infection was tested in adipose tissue and hASCs of two separate individuals. An outline of the experiment is presented below, followed by a detailed description of the techniques and assays.

Effect of Ad-36 on metabolic profile associated with insulin sensitivity. Adipose tissue explants were infected with Ad-36 or mock infected. Infection was confirmed by determining viral gene expression. D-[³H]-deoxyglucose was used to determine glucose uptake by the adipose tissue of nondiabetic and diabetic subjects. Adipogenesis was assessed by determining the time course of expressions of adipogenic genes (PPAR γ 2, aP2 [fatty acid-binding protein], lipoprotein lipase [LPL], and glycerol 3-phosphate dehydrogenase) and by counting adipocytes. Considering their important roles in glycemic control, activation of PI3K and gene expression and protein abundance of adiponectin and FAS in adipose tissue explants were determined. The gene expression assays conducted for Ad-36 infection were repeated and compared in Ad-2 versus mock-infected adipose tissue explants.

Requirement of PI3K activation for Ad-36-induced alteration in metabolic profile. Requirement of PI3K in Ad-36-induced metabolically favorable remodeling of adipose tissue was determined by using Wortmannin, a specific inhibitor of PI3K activity. The effect of Wortmannin on expressions of selected cellular genes (PPAR γ 2; aP2; LPL; platelet endothelial cell adhesion molecule [PECAM]-1, as a marker for angiogenesis induction; macrophage chemoattractant protein [MCP]-1, a marker of proinflammatory cytokine; adiponectin; FAS; and E4orf-1, a viral gene) was determined in adipose tissue explants infected with Ad-36 or mock control subjects. Finally, the effect of Wortmannin on glucose uptake by the explants was determined.

Role of Ras and insulin receptor signaling in activation of Ad-36-induced PI3K activation. Roles of two major signaling pathways in Ad-36-induced PI3K activation were determined. Phosphorylations of insulin receptor (IR), IR substrate (IRS)-1 and -2, and protein abundance of Ras were compared in explants infected with Ad-36 or mock infected. Additional experiments were conducted in hASCs to better elucidate the effect of Ad-36 on signaling pathways. The role of Ad-36 in activation of IRS-1 and PI3K was determined with and without stimulation by insulin, a well-known activator of the pathway. Finally, the requirement of Ras, another key activator of PI3K, was determined in Ad-36-induced PI3K activation and glucose uptake by hASCs. Effect of Ras knockdown on PI3K pathway activation and glucose uptake was also determined in Ad-2-infected hASCs.

Association of natural Ad-36 infection with glycemic control in humans. Considering the enhanced insulin sensitivity in rats experimentally infected with Ad-36, we hypothesized better glycemic control in humans who are

naturally infected with the virus. As a proof of concept, serum samples from nondiabetic and diabetic human subjects enrolled in unrelated studies were screened post hoc and in a blinded manner for the presence of antibodies to Ad-36. A constant-virus-decreasing-serum method was used to determine the presence of Ad-36-neutralizing antibodies as previously described (15,16). Serum neutralization assay is a gold standard and sensitive method for determining neutralizing antibodies. Due to antigenic uniqueness of Ad-36, it is highly specific for detecting Ad-36 antibodies. Available biochemical and anthropometric parameters including glucose and A1C levels were compared between the seropositive and seronegative groups.

Techniques and assays. A detailed description of the assays conducted is as follows.

Ex vivo culture of human adipose tissue explants. Before infection, explants (~100 mg for gene expression assays, ~20 mg for glucose uptake) were cultured for 3 days in DME/F-12 (no. SH30023.01; Hyclone), 1:1, plus 20% fetal bovine serum (no. SH30070.03; Hyclone) plus antibiotics/antimycotics at 37°C in 5% CO₂, unless otherwise indicated. For PI3K inhibition, 0.01 μ mol/l Wortmannin (no. W1628; Sigma) was added to cultures postinoculation.

Adipose tissue explants dedifferentiate in culture (29) and reduce adipogenic gene expression for a few days in culture. The fall in gene expression can be stabilized by supplementation of the explant media with isobutylmethylxanthine (IBMX) (29). We assessed this in two separate preliminary experiments (online appendix Fig. 1A and B [available at <http://dx.doi.org/10.2337/db07-1311>]). First, we cultured explants up to 15 days to determine the drop in gene expression and observed stabilization of PPAR γ mRNA expression after 3 days. In the second experiment, we verified if the addition of IBMX will stabilize the gene expression of explants. Expression of PPAR γ was indeed stabilized by IBMX addition. Therefore, for glucose uptake, adiponectin secretion, and Western blot assays, explant media was supplemented with 0.1 mmol/l IBMX (no. I5879; Sigma) (29). Media was replaced every 2 days. The IBMX supplementation was avoided for the gene expression experiments due to the well-known effect of IBMX on adipogenic gene expression, and the explants were inoculated with virus ~3 days after initiating incubation.

Isolation and culture of hASCs. hASCs were isolated from liposuction aspirates as previously described (30). Briefly, the stromo-vascular fraction was resuspended in DME/F-12, 1:1, plus 10% fetal bovine serum plus antibiotics/antimycotics and plated at a density of 0.156 ml of tissue digest/cm² as passage 0 (p0) and used for experiments within passages 2–4 (p2–p4). No Ad-36 DNA was detected in these tissues before experimentation.

Virus preparation. Ad-36 was obtained from the American Type Culture Collection (catalog no. VR913) and the plaque purified and propagated in A549 cells (human lung cancer cell line) as described and used previously (15,16). Ad-2 was also obtained from the American Type Culture Collection (catalog no. VR846) and propagated in A549 cells. Viral titers were determined by plaque assay (16) and cell inoculations expressed as plaque-forming units (pfu)/cell, unless otherwise indicated.

Ad-36 or Ad-2 infection of human adipose tissue explants. Explants were incubated for 1 h with 100 μ l/cm² DME/F-12, 1:1 (mock), or 100 μ l/cm² DME/F-12, 1:1, plus Ad-36 or Ad-2 (10⁶ infectious particles). Following the infection, media and virus were removed and replaced with DME/F-12, 1:1, plus 10% fetal bovine serum plus antibiotics/antimycotics.

Infection of hASCs. hASC cultures were seeded at 15,000 cells/cm². At confluency, culture media were removed and cells were incubated for 1 h with 100 μ l/cm² DME/F-12, 1:1 (mock), or DME/F-12, 1:1, plus Ad-36 or Ad-2 (2.7 pfu/cell, unless noted otherwise). Following infection, media and virus were removed and replaced with DME/F-12, 1:1, plus 20% fetal bovine serum plus antimycotics/antibiotics.

qRT-PCR. Gene expression was determined using ABI PRISM 7700 sequence detector (Applied Biosystems) and a SYBR green detection system (Bio-Rad). A standard was generated using cDNA pooled from experimental samples. Relative expression levels were determined by normalization to cyclophilin and expressed as arbitrary units.

Primers for qRT-PCR. Primers for human PECAM-1 were purchased from Applied Biosystems. All other primer sequences were as follows. Ad-36E4orf-1 forward: 5'-GGCATACTAACCCAGTCCGATG-3'; Ad-36E4orf-1 reverse: 5'-AATCACTCTCTCCAGCAGCAGG-3'; Ad-2E4orf-1 forward: 5'-cctaggcaggagggtttttc-3'; Ad-2E4orf-1 reverse: 5'-atagccgggggaatacaca-3'; human cyclophilin B forward: 5'-GGAGATGGCACAGGAGAA-3'; human cyclophilin B reverse: 5'-CGTAGT GCTTCAGTTTGAAGTTCTCA-3'; human PPAR γ 2 forward: 5'-GATACACTGTCT GCAAAACATATACAA-3'; human PPAR γ 2 reverse: 5'-CCACGGAGCT GATCCCAA-3'; human LPL forward: 5'-TATCCGCGTATTGCAGAGA-3'; human LPL reverse: 5'-AGAGAGTCGATGAAGAGATGAATGG-3'; human aP2 forward: 5'-TGTTTGGTTTTCATCCCAT-3'; human aP2 reverse: 5'-TACTGGGCCAG GAATTTGAT-3'; human G3PDH forward: 5'-CTATACAGCATCTCCAGACAA-3'; human G3PDH reverse: 5'-GGCCCTCGTAGCACACCTT-3'; human adiponectin forward: 5'-TCTGTTTCCACCCTGAC-3'; human adiponectin reverse: 5'-CAGGACGTCATCATAGAACCCTT-3'; human FAS forward: 5'-

TATGCTTCTTCGTGCAGCAGTT-3'; human FAS reverse: 5'-GCTGCCA CACGCTCCTCTAG-3'; human MCP-1 forward: 5'-CAGCCAGATGCA ATCAATGC-3'; and human MCP-1 reverse: 5'-GTGGTCCATGGAATCCTGAA-3'.

Western blot analysis. Protein concentrations were quantitated by bicinchoninic acid assay and loaded to the 4–20% or 10% polyacrylamide gel in equal amounts. Proteins then were transferred to a polyvinylidene fluoride membrane. Membranes were blocked in PBS Tween-20 containing 3% BSA and incubated with antibodies in appropriate dilutions. Signals were quantitated by GelPro 3.1 Analyzer software. Equal loading was assessed by normalization to α - β -tubulin or actin abundance. Phosphoprotein abundance was normalized to signal of total protein of interest.

Antibodies for Western blot analysis. Antibodies to Ser473 protein kinase B (PKB), total PKB, Ras, secondary antibodies, and α - β -tubulin were purchased from Cell Signaling (Danvers, MA). Antibodies to adiponectin and FAS were purchased from Abcam (Cambridge, MA). Antibodies to IR- β were purchased from Upstate (Chicago, IL). Antibodies to IRS-1 and -2 were purchased from Santa Cruz (Santa Cruz, CA).

Adipocyte counts. Adipose tissue explants were placed in histological cassettes and fixed overnight in Bouin's solution. After fixation, explants were washed with water for 2 h and placed in 70% ethanol overnight before processing. Tissues were embedded in paraffin, and 6- μ m slices were subjected to standard hematoxylin and eosin staining protocol. Adipocytes were counted using Metamorph software and adipocyte numbers expressed as cells per unit surface area. Six slices each from three individual explants were used for calculation.

Adiponectin secretion. Adiponectin was measured in the media of human adipose tissue explants cultured for 3 days postinfection by Western blotting as described above. Adiponectin levels in the media were normalized to total protein of explants and expressed as arbitrary units.

Glucose uptake assays. D-[3 H]-deoxy-glucose was purchased from Amersham (Piscataway, NJ). Glucose uptake assays were performed as previously described (31). Briefly, human adipose tissue explants (~20 mg) were incubated at 37°C, 5% CO₂ for 30 min in 500 μ l DME/F-12, 1:1, plus 2% BSA. In a pilot experiment, we determined the glucose uptake by adipose tissue explants to be linear up to 40 min of incubation (online appendix Fig. 2). For adequate tissue permeability, a 30-min incubation was used. Explants were washed three times in Krebs-Ringer HEPES buffer plus 1% BSA. Explants were incubated for 15 min in Krebs-Ringer HEPES buffer plus 1% BSA before the addition of 2 μ mol/l [3 H]-D-deoxy-glucose and 50 μ mol/l glucose to each well and further incubation for 30 min. Explants were washed three times in ice-cold Krebs-Ringer HEPES plus 1% BSA to stop the reaction and to remove unincorporated label. Explants were blotted and incubated for 30 min at 65°C in 1 N NaOH before protein quantitation, and 3 H radioactivity was determined by scintillation counting. Data are expressed as picomoles 2-deoxyglucose per milligram protein.

Ras short hairpin RNA assay. hASC cells were cultured to 80% confluency in growth media in 100-mm dishes and infected with Ad-36 (3.8 pfu/cell). Four days postinfection, Ras short hairpin RNA (shRNA) vector transfection was conducted using 20 μ g pKD-Ras-v1 plasmid (catalog no. 62-214) or pKD-neg control-v1 plasmid (catalog no. 62-002) from Upstate Biotechnology (Lake Placid, NY). The efficiency of Ras knockdown was confirmed by qRT-PCR. Four days after the shRNA transfection, glucose uptake was determined in mock or Ad-36-infected cells, Ad-36-infected cells transfected with Ras shRNA, or negative control shRNA, without insulin or after 100 nmol/l insulin stimulation for 15 min. Effect of Ras knockdown on glucose uptake and PI3K activity was determined. In a separate experiment, hASCs were infected with Ad-2, Ad-36 (10 pfu/cell), or mock infected and transfected with either pKD-Ras-v1 plasmid or pKD-neg-control-v1 plasmid. About 4 days postinfection, glucose uptake was determined and the samples were harvested for Western blot analysis.

PI3K activity assay. A total of 500 μ g of hASCs total protein extract was subjected to immunoprecipitation with 3 μ g of PI3K p85 polyclonal antiserum (Upstate) to determine PI3K activity as previously described (19). The PI3K-phospholipid product was visualized by autoradiography and quantitated by scanning densitometry with Quantity 1 1D software version 4.2.1 using Bio-Rad gel documentation.

Infectivity of hASCs. Near-confluent hASCs were infected with serial 10-fold dilutions of 100 μ l stock of viruses Ad-36 and Ad-2 (triplicate for each dilution) and overlaid with agar. Starting plaque-forming units (calculated using A5349 cells) were 6.5×10^7 per ml and 8×10^8 per ml for Ad-36 and Ad-2, respectively. Cells were fixed 8 days postinfection and stained with 4',6-diamidino-2-phenylindole dihydrochloride (catalog no. D1206; Invitrogen) and adenovirus hexon antibodies (rabbit antiserum catalog no. VR1079; American Type Culture Collection) followed by Alexa-fluor 594 goat anti-rabbit antibodies (catalog no. A-11012; Invitrogen). The number of plaques formed (as evident by cells expressing viral proteins) by Ad-36 and Ad2 were

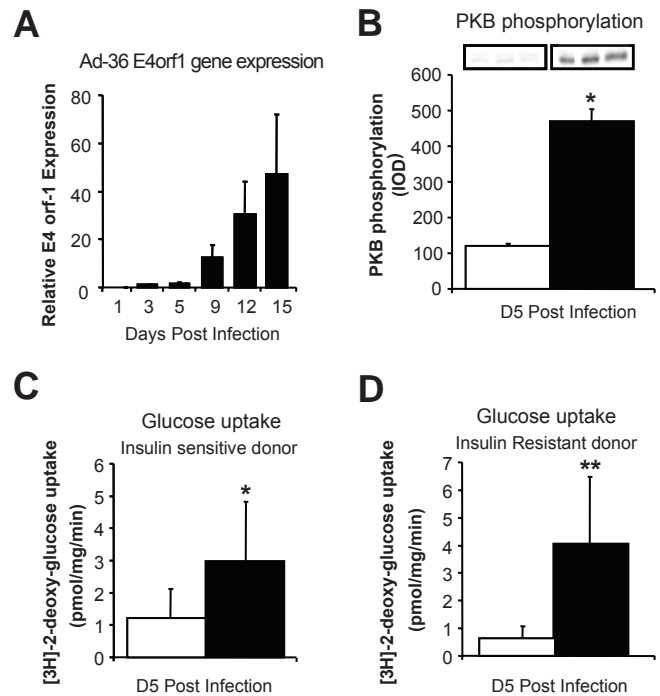


FIG. 1. Effect of Ad-36 on viral gene expression, PKB activation, and glucose uptake by human adipose tissue explants. Experiments were performed as described in RESEARCH DESIGN AND METHODS. **A:** Time course E4orf-1 gene expression after Ad-36 infection normalized to cyclophilin-B. **B:** Representative Western blot analysis of PKB phosphorylation at Ser473. Upper panel shows the autoradiograph. Lower panel shows the densitometry means \pm SE ($n = 3$ per group). \square , mock; \blacksquare , Ad-36. Values normalized to total PKB. * $P < 0.01$. **C and D:** Glucose uptake for insulin-sensitive and insulin-resistant donors. Donors were matched for BMI. Representative experiments shown as means \pm SE. **C:** Glucose uptake assay in adipose tissue from insulin-sensitive donor 5 days after Ad-36 infection. \square , mock; \blacksquare , Ad-36. **D:** Glucose uptake assay in adipose tissue from insulin-resistant (type 2 diabetic) donor 5 days after Ad-36 infection. \square , mock; \blacksquare , Ad-36. * $P = 0.036$; ** $P = 0.035$.

compared for dilutions that used similar plaque-forming units to infect (8×10^3 pfu for Ad-2 and 6.5×10^3 pfu for Ad-36).

Statistics. Assays were performed in triplicate and reported as means \pm SE. A one-sided Student's t test was used to determine significance (* $P < 0.05$). Effect of Ras shRNA on glucose uptake was determined in mock, Ad-36-, or Ad-2-infected hASCs by two-way ANOVA followed by the Tukey-Kramer test. Analyses were conducted using functions available in the base package of R (32) or SAS.

RESULTS

Ad-36 improves the metabolic profile of adipose tissue. Presence of Ad-36 E4orf-1 gene expression and its time-dependent increase confirmed successful viral entry in cells and its spread in tissue (Fig. 1A). The viral infection increased PI3K activation in the adipose tissue, as indicated by PKB phosphorylation (Fig. 1B), which probably contributed to increased glucose uptake in the adipose tissue of nondiabetic and diabetic subjects (Fig. 1C and D). Ad-36 induced the expression of early (PPAR γ 2) and late (LPL, aP2, and glycerol 3-phosphate dehydrogenase) genes of adipogenic cascade, which was maintained over 15 days postinoculation (Fig. 2A–D). Expressions of these genes did not change in the mock-infected groups. As suggested by activation of the adipogenic cascade, Ad-36-infected explants significantly increased the number of adipocytes by 21 days postinoculation (Fig. 2E–G). Ad-36-infected adipose tissue also showed greater mRNA expression and protein abundance of adiponectin and FAS (Fig. 3A–D). Thus, in human adipose tissue, Ad-36 activates adipogenesis, enhances

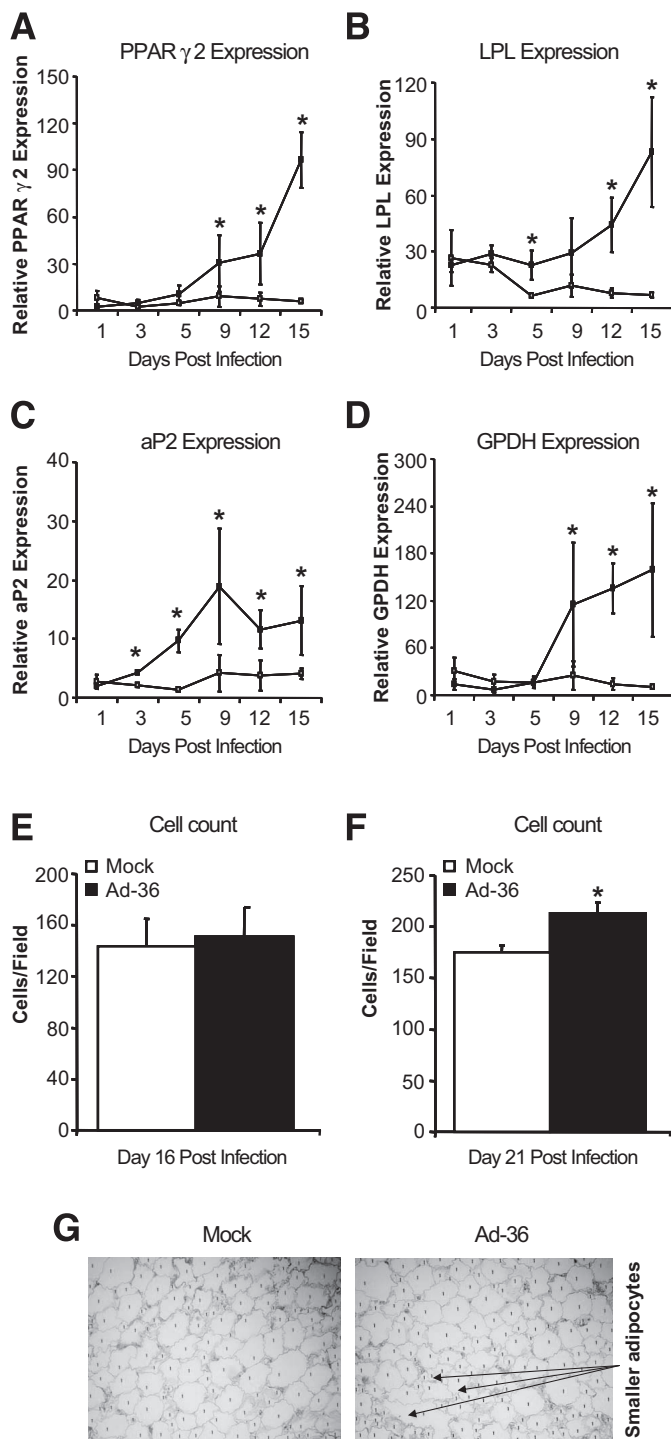


FIG. 2. Increased adipogenic gene expression and adipocyte cell numbers in Ad-36-infected human adipose tissue explants. Experiments were performed as described in RESEARCH DESIGN AND METHODS. All experiments were representative ($n = 3$ per group). Data are means \pm SE. **A–D:** Time course gene expressions measured by qRT-PCR and normalized to cyclophilin. \square , mock; \blacksquare , Ad-36. **E and F:** Number of cells per field; $n = 3$ (explants) per group. Six slices per explant were used for calculations. \square , mock; \blacksquare , Ad-36. $*P < 0.05$. **G:** Representative sections of adipose tissue used for calculation of the number of cells in **E** and **F**. Sections from Ad-36-infected explants show a greater number of smaller cells on day 21 postinoculation. Red dots indicate cells counted as adipocytes.

FAS and adiponectin, and increases glucose disposal, indicating improved metabolic profile of the tissue.

The effect of Ad-36 on adipose tissue cannot be attributed simply to any viral infection. Ad-2, another human

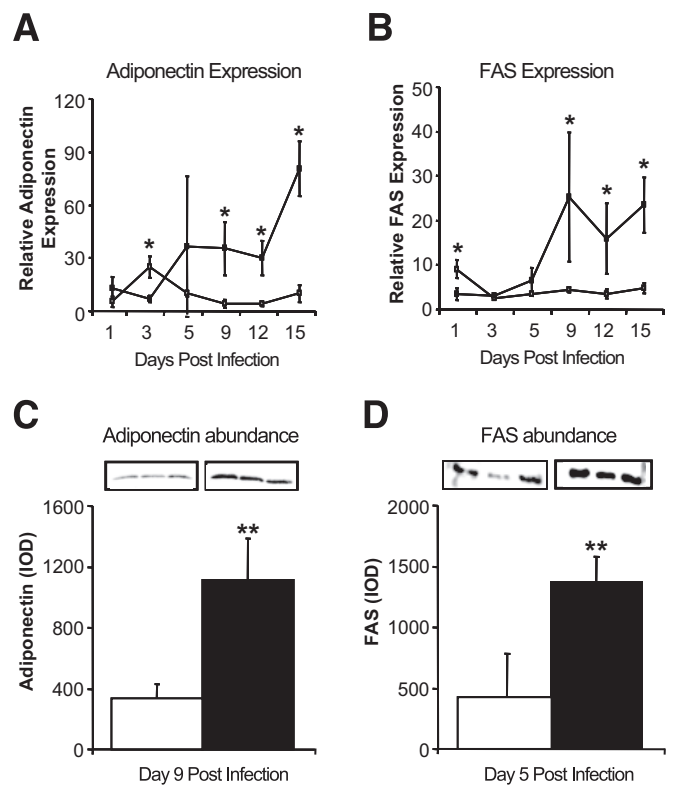


FIG. 3. Increased adiponectin and FAS in Ad-36 infection of human adipose tissue. Experiments were performed as described in RESEARCH DESIGN AND METHODS. **A and B:** Time course gene expression after Ad-36 infection as measured by qRT-PCR and normalized to cyclophilin-B. Data are means \pm SE. Representative experiment ($n = 3$ explants per group). \square , mock; \blacksquare , Ad-36. **C and D:** Western blot analysis for adiponectin (media) and FAS protein levels. \square , mock; \blacksquare , Ad-36. **C: Upper panel:** Autoradiograph of Western blot. **Lower panel:** Densitometry means \pm SE ($n = 3$ per group). Adiponectin protein levels were normalized to total protein content of individual explants. **D: Upper panel:** shows autoradiograph. **Lower panel:** Densitometry means \pm SE ($n = 3$ per group). FAS protein levels were normalized to β -tubulin. $*P < 0.05$; $**P < 0.01$.

adenovirus, which is nonadipogenic in vivo and in vitro (23,33), was used as a negative control. In addition, we conducted a plaque-forming unit assay in hASCs. The number of plaques formed in hASCs by nearly equal number of particles of the two viruses were not significantly different ([means \pm SD] Ad-36vsAd-2 13.7 ± 0.6 vs. 8.7 ± 3.2 ; $P = 0.12$). Collectively, this indicated that the difference in Ad-36 and Ad-2 in glucose uptake and other adipogenic effects is not due to their differential ability to infect human adipose tissue or hASCs. Despite successful infection of the explants as evident from viral mRNA expression (Fig. 4A), unlike Ad-36, Ad-2 did not induce expression of the above-described genes of adipogenic cascade (Fig. 4B–G). Therefore, subsequently, we focused only on the effect of Ad-36.

PI3K is required for Ad-36-induced improvement in metabolic profile. PI3K is a key molecular for several cellular pathways, which is evident from the fact that its inhibition by Wortmannin reduced the expressions of aP2, PECAM-1, adiponectin, FAS, and MCP-1 (Fig. 5A). Also, Ad-36 E4orf-1 gene expression requires PI3K activation (Fig. 5B). Furthermore, Ad-36-induced expression of PPAR γ 2, aP2, LPL, and PECAM-1 in the adipose tissue was dependent on PI3K activation (Fig. 5C–F). Via PI3K activation, Ad-36 suppressed expression of MCP-1, a proinflammatory marker. Wortmannin reversed Ad-36-induced MCP-1 suppression, which also showed that its effect on

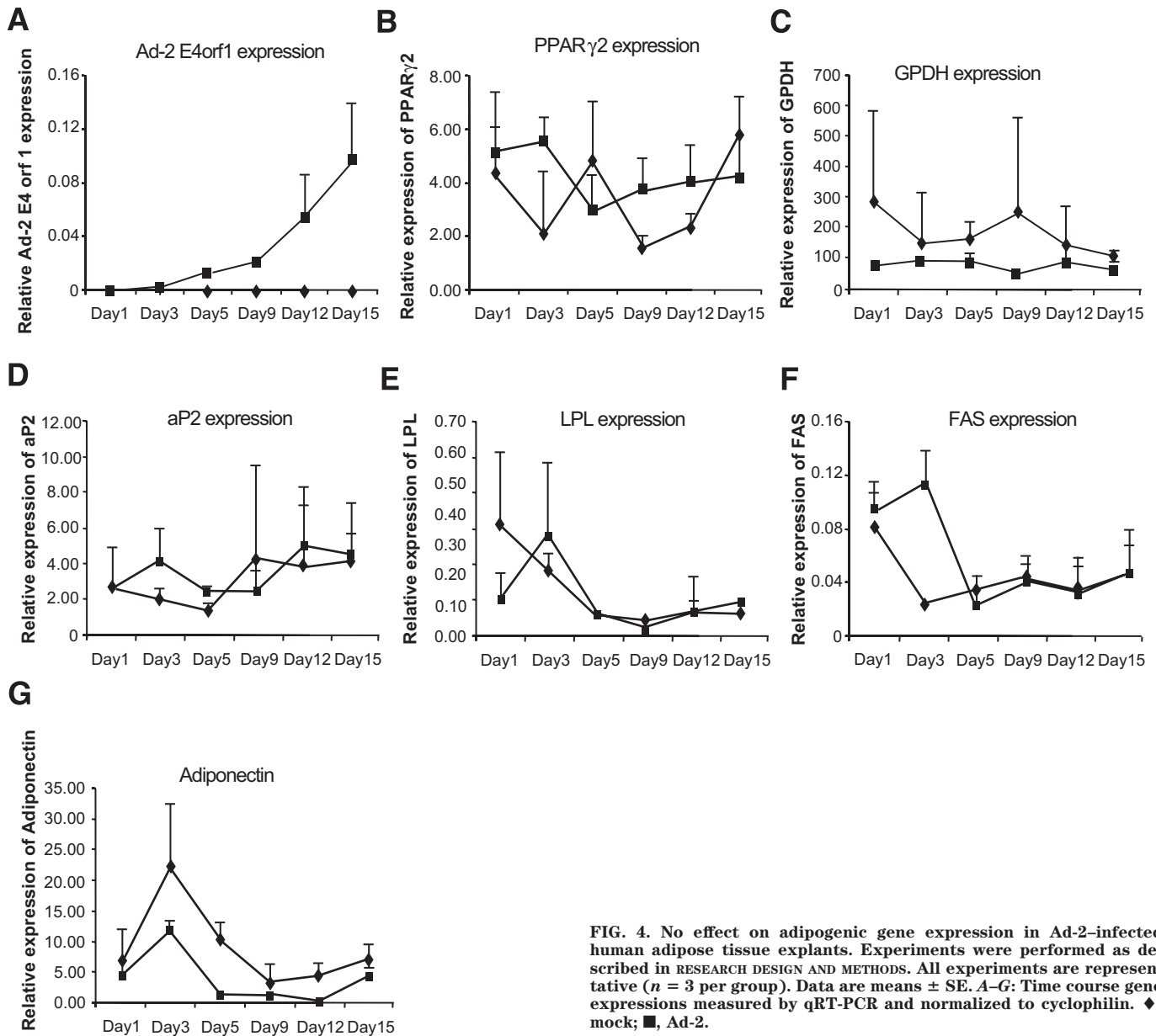


FIG. 4. No effect on adipogenic gene expression in Ad-2-infected human adipose tissue explants. Experiments were performed as described in RESEARCH DESIGN AND METHODS. All experiments are representative ($n = 3$ per group). Data are means \pm SE. A–G: Time course gene expressions measured by qRT-PCR and normalized to cyclophilin. ♦, mock; ■, Ad-2.

cellular gene expression is not universally inhibitory in the presence of the virus. Importantly, Ad-36 required PI3K to enhance glucose uptake (Fig. 5G). Collectively, these results demonstrated that Ad-36 induces metabolic changes in adipose tissue via activation of PI3K.

Ras, but not insulin receptor signaling, is required for Ad-36-induced PI3K activation and glucose uptake. The effect of Ad-36 on insulin receptor signaling and Ras signaling, the two key activators of PI3K, was determined in adipose tissue explants and in hASCs. Ad-36 reduced the abundance and activation of IR, IRS-1, and IRS-2 in explants but greatly increased Ras protein abundance in explants (Fig. 6A), suggesting that the virus uses Ras signaling rather than insulin receptor signaling for PI3K activation. In hASCs, under basal or insulin-stimulated conditions, Ad-36 activated PKB phosphorylation but blocked IRS-1 tyrosine phosphorylation (Fig. 6B–E), confirming its lack of contribution in Ad-36-induced PI3K signaling. Therefore, we tested the participation of Ras signaling in Ad-36-induced activation of the PI3K path-

way. As hypothesized, Ras-specific shRNA abrogated Ad-36-induced PI3K activity (Fig. 7A), indicating Ras as an upstream regulator of Ad-36-induced PI3K activity. Furthermore, as predicted from its role in Ad-36-induced PI3K activation, knockdown of Ras expression by shRNA showed that Ad-36-induced glucose uptake by hASCs is Ras dependent (Fig. 7B). Interestingly, Ad-36 induced a fourfold increase in glucose uptake by hASCs, which could not further be enhanced by insulin stimulation (Fig. 7B), which further demonstrated the robust and insulin-independent effect of Ad-36 on glucose uptake.

Finally, we compared the effects of Ad-36 and Ad-2 on glucose uptake in hASCs. Compared with the mock-infected hASCs, Ad-36, but not Ad-2, increased glucose uptake by ~ 2.5 -fold (Fig. 7C), and Ras abundance knockdown was up to 50% (Fig. 7D). As expected, the Ad-36 group showed greater PI3K pathway activation, as indicated by increased Ser-473 and Thr-308 phosphorylation of Akt/PKB. Ras knockdown reduced Ad-36 protein abundance and Akt/PKB phosphorylation (Fig. 7D), and Ad-2

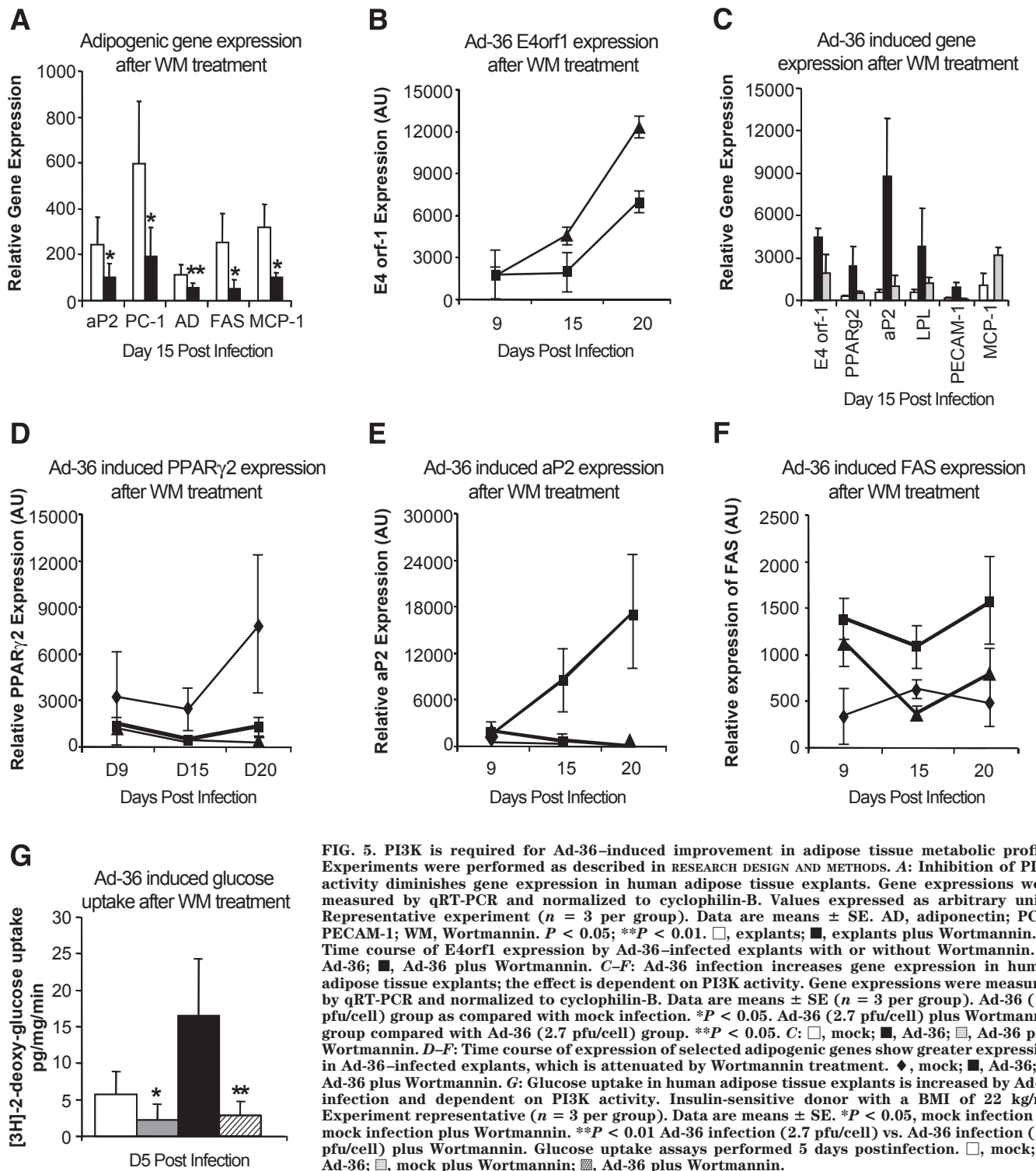


FIG. 5. PI3K is required for Ad-36-induced improvement in adipose tissue metabolic profile. Experiments were performed as described in RESEARCH DESIGN AND METHODS. **A:** Inhibition of PI3K activity diminishes gene expression in human adipose tissue explants. Gene expressions were measured by qRT-PCR and normalized to cyclophilin-B. Values expressed as arbitrary units. Representative experiment ($n = 3$ per group). Data are means \pm SE. AD, adiponectin; PC-1, PECAM-1; WM, Wortmannin. $P < 0.05$; $**P < 0.01$. □, explants; ■, explants plus Wortmannin. **B:** Time course of E4orf1 expression by Ad-36-infected explants with or without Wortmannin. ▲, Ad-36; ■, Ad-36 plus Wortmannin. **C–F:** Ad-36 infection increases gene expression in human adipose tissue explants; the effect is dependent on PI3K activity. Gene expressions were measured by qRT-PCR and normalized to cyclophilin-B. Data are means \pm SE ($n = 3$ per group). Ad-36 (2.7 pfu/cell) group as compared with mock infection. $*P < 0.05$. Ad-36 (2.7 pfu/cell) plus Wortmannin group compared with Ad-36 (2.7 pfu/cell) group. $**P < 0.05$. **C:** □, mock; ■, Ad-36; ▨, Ad-36 plus Wortmannin. **D–F:** Time course of expression of selected adipogenic genes show greater expression in Ad-36-infected explants, which is attenuated by Wortmannin treatment. ◆, mock; ■, Ad-36; ▲, Ad-36 plus Wortmannin. **G:** Glucose uptake in human adipose tissue explants is increased by Ad-36 infection and dependent on PI3K activity. Insulin-sensitive donor with a BMI of 22 kg/m². Experiment representative ($n = 3$ per group). Data are means \pm SE. $*P < 0.05$, mock infection vs. mock infection plus Wortmannin. $**P < 0.01$ Ad-36 infection (2.7 pfu/cell) vs. Ad-36 infection (2.7 pfu/cell) plus Wortmannin. Glucose uptake assays performed 5 days postinfection. □, mock; ■, Ad-36; ▨, mock plus Wortmannin; ▩, Ad-36 plus Wortmannin.

did not increase Ras abundance, PKB activation, or glucose uptake. Ad-2 protein abundance was not affected by Ras knockdown.

Natural infection of Ad-36 is associated with better glycemic control in humans. Blinded post hoc screening for neutralizing antibodies to Ad-36 in nondiabetic as well as diabetic subjects enrolled in unrelated studies showed ~12% prevalence of Ad-36 infection (Table 1). Study 1 included 3 men and 34 women who were nondiabetic or diabetic (31 Caucasians and 6 African Americans) (Table 1A). Study 2 included 16 men and 32 female diabetic

subjects (30 Caucasians and 18 African Americans) (Table 1B). Seropositive and seronegative individuals were not significantly different with respect to age, BMI, and blood pressure in respective studies. However, seropositivity was a significant predictor of lower fasting glucose (Table 1A; online appendix Fig. 3) or A1C levels (Table 1B; online appendix Fig. 4). Moreover, within the diabetic group, Ad-36 seropositivity was significantly associated with lower serum cholesterol and LDL cholesterol levels (Table 1B). Although not causal, these findings are remarkably similar to the virus-induced phenotypic patterns

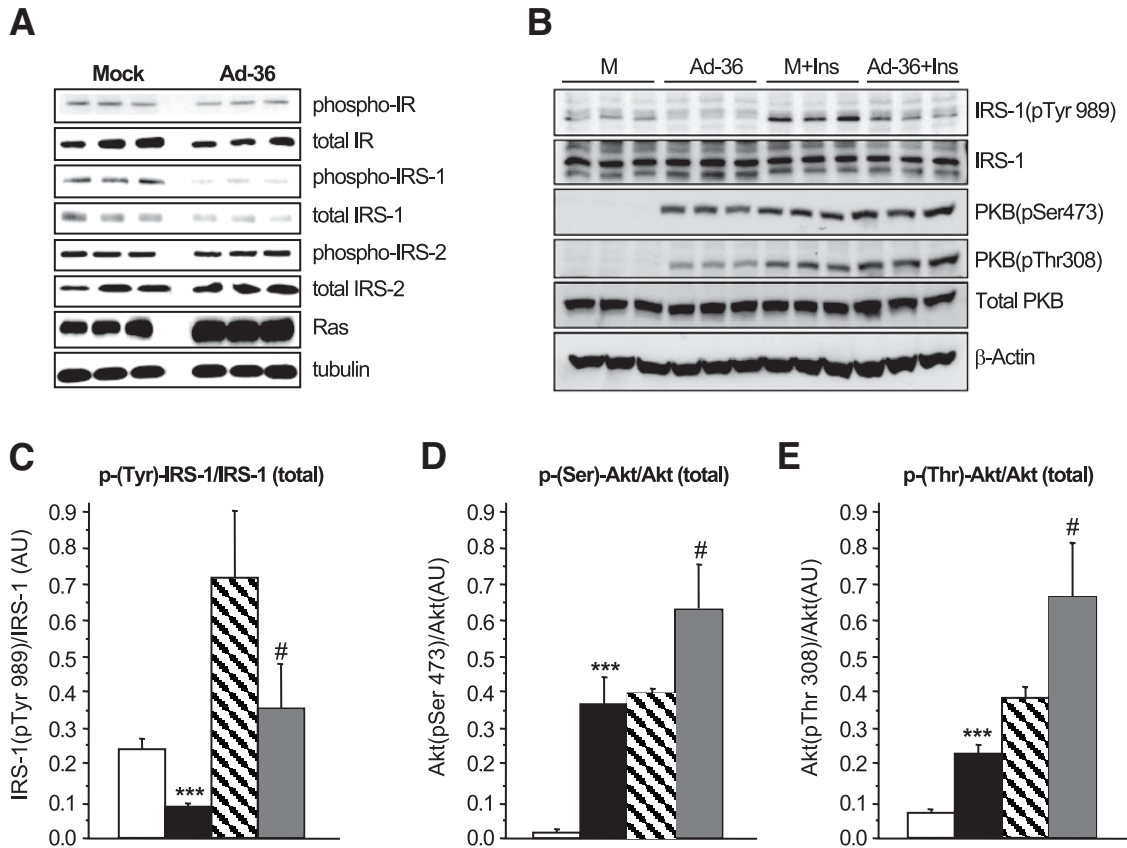


FIG. 6. Effect of Ad-36 on cell signaling involved in glucose uptake. Experiments were performed as described in RESEARCH DESIGN AND METHODS. **A:** Western blot analysis shows that Ad-36 decreased insulin signaling proteins and their phosphorylations and increased Ras protein levels in adipose tissue explants. **B–E:** Representative Western blot analysis of total protein extracts of hASCs that were mock or Ad-36 infected in insulin-stimulated and basal conditions. * $P < 0.05$ Ad-36 infection (2.7 pfu/cell) vs. mock. ** $P < 0.01$, $P < 0.001$. Experiments were conducted on three different patients. Densitometry means \pm SD ($n = 3$ per group). □, mock; ■, Ad-36; ▨, mock plus insulin; ▩, Ad-36 plus insulin. **B:** Membranes were incubated with antibodies to IRS-1 (pTyr 989), IRS-1, PKB (pSer473), PKB (pThr308), PKB, or β -actin. **C:** Densitometric analysis of IRS-1 (pTyr 989) abundance. **D:** Densitometric analysis of PKB (pSer473) abundance. **E:** Densitometric analysis of PKB (pThr308) abundance.

observed in experimentally infected animals (18) and suggest that natural Ad-36 infection may be an important moderator of glucose disposal in humans.

DISCUSSION

Adipose tissue is comprised various cell types, including those of adipogenic, endothelial, and immune lineage. Therefore, adipose tissue explants were mainly used for better representation of the collective response of the constituent cell types (31,34,35). The use of explants allowed for the testing of the potential of Ad-36 to favorably alter the metabolic profile of the entire tissue, including the proadipogenic, angiogenic, and anti-inflammatory effects. A limitation of explant use is their limited utility to study cell signaling by methods such as RNA knockdown. Therefore, hASCs were used to further elucidate molecular mechanism.

Overall, Ad-36 improves the metabolic profile of human adipose tissue, as indicated by greater glucose uptake; reduced expression of MCP-1, a proinflammatory marker; increased FAS and adiponectin levels; and increased expression of adipogenic genes such as PPAR γ 2, which are associated with better glucose disposal (6,36). This effect of the virus very closely resembles a transgenic mouse model, which shows improved metabolic profile due to adipose tissue expansion through adiponectin overexpression (14). Ad-36-induced glucose uptake is particularly robust, even in basal conditions, which could not be enhanced

further by insulin stimulation (Fig. 7B), indicating maximal stimulation of the glucose uptake process by the virus. It is particularly noteworthy that Ad-36 increased the glucose uptake by adipose tissue of even a diabetic subject, suggesting its insulin-independent effect. We reported increased and insulin-independent glucose uptake in human primary skeletal muscle cells infected with Ad-36 (37). Ability of Ad-36 to enhance glucose uptake by adipose tissue and skeletal muscle may collectively contribute to enhanced glucose disposal observed in experimental (18) and natural (Table 1) Ad-36 infection. The cellular targets of Ad-36 action need to be identified to harness this potential of the viral proteins for therapeutic use.

Ad-36 influences adipose tissue metabolic profile in a PI3K-dependent manner. The activation of the PI3K pathway induces cell proliferation and adipogenesis (38,39), increases adiponectin expression (40), reduces inflammatory response (41), and enhances glucose uptake (42) in adipocytes and participates in a number of other cell functions. The pivotal role of PI3K in cellular metabolism was also evident from the reduction in cellular gene expression in the presence of Wortmannin (Fig. 5A and B). PI3K activation is also required for cellular entry by some adenoviruses (43,44), but not all (45), and human adenoviruses such as Ad-5, Ad-9, and Ad-19 are known to activate PI3K (45–48). Since the adipose tissue was infected with Ad-36 before Wortmannin treatment, the effect

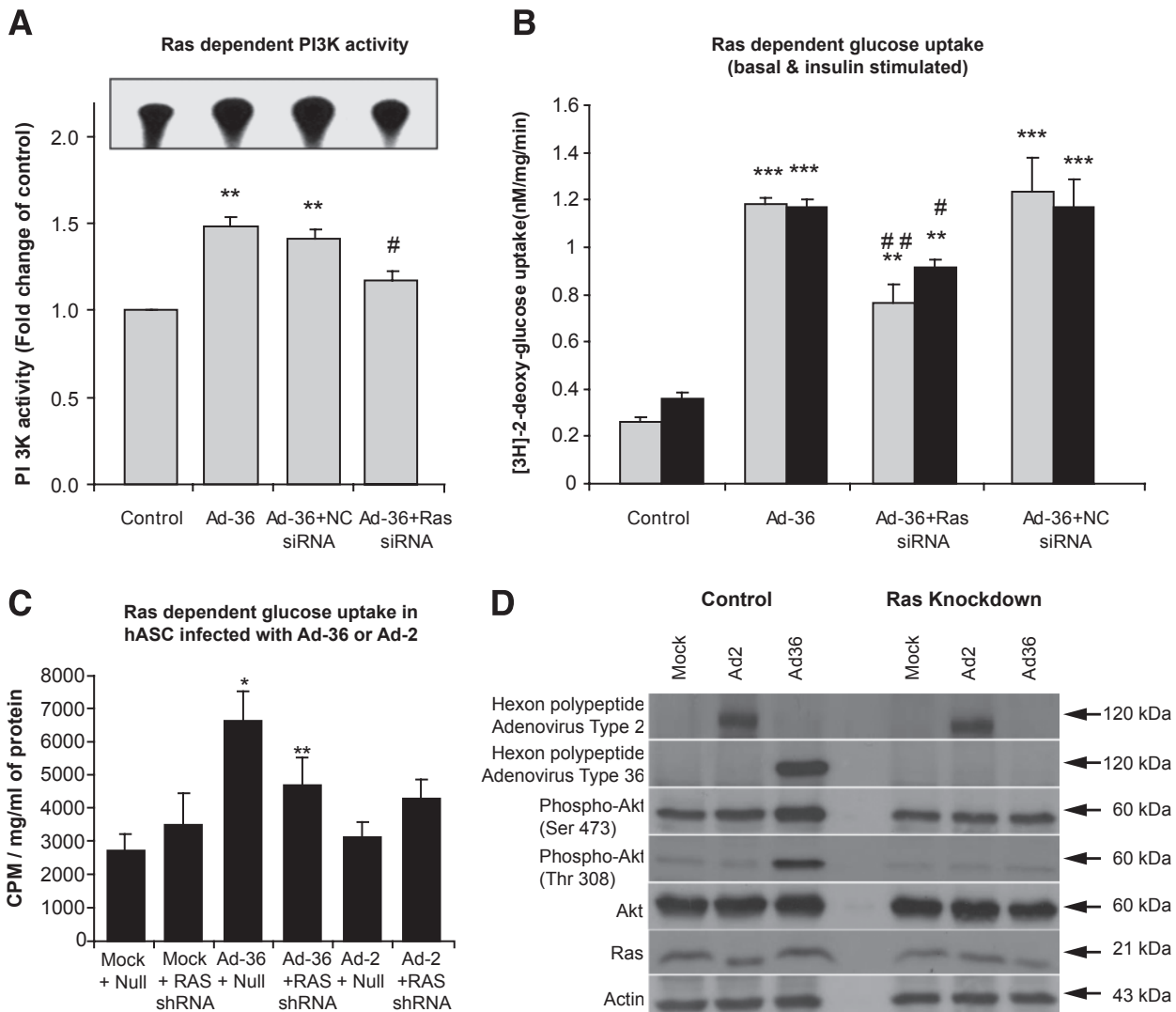


FIG. 7. Effect of Ras siRNA transfection on glucose uptake in Ad-36-infected hASCs. Three days after Ad-36 infection (3.8 pfu/cell), hASCs were transfected with 2 μ g/ml Ras siRNA mixed with twofold SatisFfection transfection reagent (Stratagene, La Jolla, CA). Three days later, PI3K activation was determined by thin-layer chromatography, and glucose uptake was determined in the absence and presence of insulin (100 nmol/l insulin was added during the last 15 min of incubation). Data are means \pm SE. **A:** Ad-36 induced PI3K activation with or without Ras siRNA transfection. ** $P < 0.01$ Ad-36 vs. control; # $P < 0.01$ Ad-36 vs. Ad-36 plus Ras siRNA. **B:** Ad-36 induced glucose uptake with or without Ras siRNA transfection. ** $P < 0.01$; *** $P < 0.01$ Ad-36 vs. control; # $P < 0.05$; ## $P < 0.01$ Ad-36 vs. Ad-36 plus siRNA. □, basal; ■, insulin. **C:** hASCs were mock infected or infected with Ad-36 or Ad-2 as described in RESEARCH DESIGN AND METHODS and transfected with null vector or a RAS-specific shRNA vector. Compared with the “mock + null vector” group, “Ad-36 + null vector” group but not “Ad-2 + null vector” had greater glucose uptake ($P < 0.0001$). Ras shRNA significantly altered the glucose uptake only in the Ad-36-infected group (** $P < 0.05$). **D:** Proteins harvested from samples of seven from **C** were used for Western blots. Representative blots from a triplicate sample are presented.

of PI3K inhibition on cellular entry of Ad-36 was not relevant.

Insulin is a well-known activator of PI3K activity. The binding of insulin to the insulin receptor activates insulin receptor’s internal tyrosine kinase activity. The activated tyrosine-phosphorylated insulin receptor phosphorylates IRS, which in turn activates PI3K, eventually leading to Glut4 translocation and glucose uptake. While IRS-1 is particularly important for insulin-stimulated PI3K activity and Glut4 translocation in adipose cells (49), Ras signaling also plays a prominent role in activation of PI3K (50,51) and glucose uptake (52). Therefore, the roles of insulin and Ras signaling were determined in Ad-36-induced PI3K activation.

Ad-36 blocked IRS-1 activation by insulin in hASCs, perhaps due to negative feedback from activated PI3K, as shown by Tanti et al. (53) and reviewed by Ye (54). Therefore, enhanced activation of PI3K signaling by insu-

lin in the presence of the virus is unlikely to be IRS-1 mediated. Instead, it is probably mediated via the Ras signaling pathway, as shown by Sakaue et al. (55), which showed that tyrosine phosphorylation of IR by insulin activates Ras via son of sevenless (SOS), a guanine nucleotide exchange protein, and such activation is independent of IRS-1 activation.

Ad-36-induced glucose uptake as well as the viral gene expression appears to be dependent on PI3K and Ras, which are also recruited by various other viruses for their replication (43,56–58). It is unclear if Ad-36 requires Ras or PI3K for directly inducing the downstream glucose uptake or indirectly for viral gene expression itself. Our data suggest that either or both possibilities may exist. Ad-36 may upregulate the Ras-PI3K pathway to increase viral gene expression, which in turn, may increase glucose uptake via either the Ras-PI3K pathway or another unknown mechanism. To clarify this further, future work

TABLE 1
Association of Ad-36 infection with glycemic control in humans

	Ad-36 positive	Ad-36 negative	<i>P</i>
A. Ad-36 increases glucose uptake in human skeletal muscle cells			
<i>n</i> (%)	5 (13.5)	32 (86.5)	
Age (years)	46.7 ± 11	49.1 ± 9	NS
BMI (kg/m ²)	43.6 ± 4.6	47.4 ± 5.3	NS
Systolic blood pressure (mmHg)	125 ± 17	129 ± 18	NS
Diastolic blood pressure (mmHg)	79 ± 14	82 ± 10	NS
Glucose (mg/dl)	74 ± 29	124 ± 53	0.02
B. Ad-36 seropositivity in diabetic subjects			
<i>n</i> (%)	6 (12.5)	42 (87.5)	
Age (years)	67 ± 10	60 ± 17	NS
BMI (kg/m ²)	34.6 ± 5	35.9 ± 6	NS
Waist circumference (cm)	106.2 ± 9	111.6 ± 14	NS
Weight (kg)	88 ± 9	98 ± 16	NS
Percent body fat (%)	42.3 ± 4	39.5 ± 9	NS
Visceral adipose tissue (kg)	6.0 ± 0.6	6.7 ± 2	NS
Systolic blood pressure (mmHg)	130 ± 17	125 ± 16	NS
Diastolic blood pressure (mmHg)	79 ± 8	78 ± 9	NS
Total cholesterol (mg/dl)	167 ± 31	199 ± 34	0.02
LDL cholesterol (mg/dl)	78 ± 37	111 ± 27	0.04
A1C (%)	5.7 ± 0.4	6.3 ± 0.8	0.005

Data are means ± SD unless otherwise indicated.

should determine whether Ras or PI3K knockdown will attenuate Ad-36–induced glucose uptake, when the viral gene or protein expression is maintained by a different promoter.

Human adenoviruses are known to cooperate with Ras in cell transformation (59) and to activate PI3K via Ras signaling (47) and increase glucose uptake in vitro (60). As predicted, Ras was required for Ad-36–induced activation of PI3K and the consequential increase in glucose uptake in basal as well as insulin-stimulated glucose uptake. Thus, Ras signaling appears to be a key event in Ad-36–induced increase in glucose uptake by adipose tissue. This assertion is supported by an earlier study (52) that showed that selective overexpression of Ras in adipose tissue increased glucose uptake by the tissue and improved whole-body insulin sensitivity of the transgenic mice.

Although like Ad-36, other human adenoviruses activate Ras and PI3K signaling pathways, which may then increase glucose uptake in vitro, their ability to modulate the metabolic profile of adipose tissue is unknown. For instance, unlike Ad-36, human adenoviruses Ad-2 and Ad-31 do not show causation or association with adiposity in animals and humans, respectively (33,61). We showed that Ad-2 does not increase RAS abundance or glucose uptake in vitro (Fig. 7C and D). Ad-9 upregulates PI3K via Ras signaling (47) and enhances adipogenesis in vitro (22), but its effect on glucose disposal is unknown. Characteristics unique to Ad-36 that help it increase adipogenesis and glucose uptake are unclear.

Effect of human adenoviruses on proinflammatory cytokines is varied. Ad-36 reduced MCP-1 expression in explants, but Ad-19 increases MCP-1 expression (62) and Ad-7 increases interleukin-8 production (63). On the other hand, adenovirus types 16, 35, and 37 reduce proinflammatory cytokine expressions (64). Interestingly, Ad-37 is reported to increase adiposity in animals (33). Overall, unlike Ad-36, other adenoviruses have not been tested comprehensively for their effects on adipogenic pathways and glucose metabolism. Recognizing the ability of Ad-36 to modulate metabolic profile of adipose tissue should

provide impetus to evaluate other human adenoviruses for similar potential.

Increased adiposity is associated with lower adiponectin levels and greater inflammation and insulin resistance, and reduction in adipose tissue mass reverses the changes (65–69). However, reduction of adipose tissue and preventing its regain are challenging. Our findings indicate that akin to some therapeutic agents (5,11), it is possible to induce a metabolically favorable profile in the adipose tissue, without a reduction in adiposity. This is a potentially important finding for eventually developing novel strategies to manage adiposity-induced glucose dysregulation. Further studies are required to identify the viral protein responsible for the effect and to elucidate its interaction with cellular proteins participating in tissue remodeling.

Finally, the association of natural Ad-36 infection with better glycemic control in diabetic and nondiabetic human subjects provides a proof of concept that is expected to offer a human relevance to the main observations described. The number of Ad-36 seropositive subjects is relatively small in these studies, and a dedicated larger prospective study with human samples better characterized for glucose metabolism is required to further test the association. Nevertheless, it is interesting that even with a small number of Ad-36–positive subjects, this study conducted in a blinded manner yielded 40% lower glucose levels (Table 1A), 16% lower serum cholesterol, 30% lower LDL cholesterol, and 10% lower A1C levels (Table 1B) in Ad-36–seropositive groups.

Better glycemic control in Ad-36–infected animals was discovered recently (18). Therefore, a seropositivity-dependent difference, if any, in glucose values was not determined in our earlier study that showed Ad-36 seropositivity in 30% of obese and 11% of the nonobese subjects screened (70). We revisited unpublished data of this study (R.L. Atkinson, B.A. Israel, A.S. Augustus, N.V. Dhurondhor, unpublished data). Fasting glucose measurements were available for 85 obese subjects (73 seronegative and 12 seropositive for Ad-36) recruited

from Wisconsin. The fasting blood glucose levels were lower for the Ad-36-seropositive group (102 ± 36 vs. 95 ± 7.6 ; $P = 0.06$).

Better glycemic control in Ad-36-infected subjects is intriguing. Ad-36-neutralizing antibody titer was not high enough to suggest an acute infection. This indicates a longer-lasting change in systemic glucose handling following a natural infection in these subjects. It is noteworthy that Ad-36 increases adiposity in experimentally infected animals and improves glycemic control, but only the later was observed to be associated with Ad-36 infection in these subjects. Considering the numerous other adipogenic stimuli in humans, it is possible that the Ad-36-negative subjects gained adiposity due to other causes. We postulate that the Ad-36-induced expansion of adipose tissue is accompanied by improvement in metabolic profile of the tissue, compared with a similar degree of adiposity acquired due to other reasons.

Although Ad36 remarkably increases adipose tissue-glucose uptake independent of insulin in vitro, the virus does not cause uncontrolled glucose uptake and hypoglycemia in experimentally infected rats (18) or humans naturally infected with Ad-36. Perhaps this is because the in vivo physiological regulatory controls of circulating glucose homeostatic mechanisms prevent uninhibited glucose clearance and consequential fasting hypoglycemia. Moreover, Ad-36 induces glucose uptake in a virus dose-dependent manner (37). Therefore, the degree of infectivity may be another determinant of the magnitude of glucose uptake induced by the virus in vivo. It is likely that due to immune response, tissue accessibility, or other factors, a relatively limited fraction of cells are infected in an organism. Hence, the net result of contributions from infected and uninfected cells may balance out the robust effect of Ad-36 on cellular glucose uptake observed in vitro to a more moderate effect without severe hypoglycemia in vivo.

We previously reported that Ad-36 increases the commitment of hASCs to adipogenic lineage (71). Moreover, similar to its effect on adipose tissue, Ad-36 also increases glucose uptake by human skeletal muscle (37) in a Ras-mediated, PI3K-dependent manner. Ad-36 increases FAS levels, suggesting the conversion of increased cellular glucose to lipids via de novo lipogenic pathway. Taken together, we postulate that Ad-36 expands adipose tissue and increases glucose uptake in skeletal muscle and adipose tissue, which collectively leads to better glycemic control in vivo. By further identifying the viral proteins and their cellular targets involved in the effect, novel therapeutic agents may be developed for enhancing glucose disposal in type 1 or type 2 diabetes and for improving the adipose tissue metabolic profile associated with insulin resistance.

ACKNOWLEDGMENTS

This study was supported in part by funds from the National Institutes of Health (NIH R-01DK066164 [to N.V.D.] and P50AT002776-01 and R01DK060126 [to W.T.C.]). We gratefully acknowledge that human adipose tissue and isolated hASCs were provided by Dr. Jeffery M. Gimble of the Molecular Mechanisms Core of the Pennington Biomedical Research Center Clinical Nutrition Research Unit (NIH 1P30DK072476).

REFERENCES

- Oh DK, Ciaraldi T, Henry RR: Adiponectin in health and disease. *Diabetes Obes Metab* 9:282–289, 2007
- Bastard JP, Lagathu C, Maachi M, et al.: [Adipose tissue cytokines and insulin resistance]. *J Annu Diabetol Hotel Dieu* 29–37, 2004 [Article in French]
- Capeau J, Magre J, Lascols O, et al.: Diseases of adipose tissue: genetic and acquired lipodystrophies. *Biochem Soc Trans* 33:1073–1077, 2005
- Ranganathan G, Unal R, Pokrovskaya I, et al.: The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. *J Lipid Res* 47:2444–2450, 2006
- de Souza CJ, Eckhardt M, Gagen K, et al.: Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* 50:1863–1871, 2001
- Tarcin O, Bajaj M, Akalin S: Insulin resistance, adipocyte biology and thiazolidinediones: a review. *Metab Syndr Relat Disord* 5:103–115, 2007
- Standaert ML, Kanoh Y, Sajan MP, et al.: Cbl, IRS-1, and IRS-2 mediate effects of rosiglitazone on PI3K, PKC-lambda, and glucose transport in 3T3/L1 adipocytes. *Endocrinology* 143:1705–1716, 2002
- Todd MK, Watt MJ, Le J, et al.: Thiazolidinediones enhance skeletal muscle triacylglycerol synthesis while protecting against fatty acid-induced inflammation and insulin resistance. *Am J Physiol Endocrinol Metab* 292:E485–E493, 2007
- van Doorn M, Kemme M, Ouwens M, et al.: Evaluation of proinflammatory cytokines and inflammation markers as biomarkers for the action of thiazolidinediones in type 2 diabetes mellitus patients and healthy volunteers. *Br J Clin Pharmacol* 62:391–402, 2006
- Yu JG, Javorschi S, Hevener AL, et al.: The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 51:2968–2974, 2002
- Hu X, Feng Y, Liu X, et al.: Effect of a novel non-thiazolidinedione peroxisome proliferator-activated receptor alpha/gamma agonist on glucose uptake. *Diabetologia* 50:1048–1057, 2007
- Smith SR, Xie H, Baghian S, et al.: Pioglitazone changes the distribution of adipocyte size in type 2 diabetes. *Adipocytes* 2:11–22, 2006
- Medina-Gomez G, Gray SL, Yetukuri L, et al.: PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genet* 3:e64, 2007
- Kim JY, van de Wall E, Laplante M, et al.: Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 117:2621–2637, 2007
- Dhurandhar NV, Israel BA, Kolesar JM, et al.: Transmissibility of adenovirus-induced adiposity in a chicken model. *Int J Obes Relat Metab Disord* 25:990–996, 2001
- Dhurandhar NV, Israel BA, Kolesar JM, et al.: Increased adiposity in animals due to a human virus. *Int J Obes Relat Metab Disord* 24:989–996, 2000
- Dhurandhar NV, Whigham LD, Abbott DH, et al.: Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys. *J Nutr* 132:3155–3160, 2002
- Pasarica M, Shin AC, Yu M, et al.: Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity (Silver Spring)* 14:1905–1913, 2006
- Vangipuram SD, Sheele J, Atkinson RL, et al.: A human adenovirus enhances preadipocyte differentiation. *Obes Res* 12:770–777, 2004
- Rathod M, Vangipuram SD, Krishnan B, et al.: Viral mRNA expression but not DNA replication is required for lipogenic effect of human adenovirus Ad-36 in preadipocytes. *Int J Obes (Lond)* 31:78–86, 2007
- Rathod MA, Rogers PM, Dhurandhar NV: Human adenovirus Ad-36 infection induces differentiation and replication of preadipocytes. *Obes Rev* 7:138, 2006
- Vangipuram SD, Yu M, Tian J, et al.: Adipogenic human adenovirus-36 reduces leptin expression and secretion and increases glucose uptake by fat cells. *Int J Obes (Lond)* 31:87–96, 2007
- Rogers PM, Fusinski K, Rathod MA, et al.: Human adenovirus Ad-36 induces adipogenesis via its E4 orf-1 gene. *Int J Obes* 32:397–406, 2007
- Hanlon GW: Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *Int J Antimicrob Agents* 30:118–128, 2007
- Bischoff JR, Kirn DH, Williams A, et al.: An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274:373–376, 1996
- Crompton AM, Kirn DH: From ONYX-015 to armed vaccinia viruses: the education and evolution of oncolytic virus development. *Curr Cancer Drug Targets* 7:133–139, 2007
- Pan Q, Liu B, Liu J, et al.: Synergistic induction of tumor cell death by

- combining cisplatin with an oncolytic adenovirus carrying TRAIL. *Mol Cell Biochem* 304:315–323, 2007
28. Libertini S, Iacuzzo I, Ferraro A, et al.: Lovastatin enhances the replication of the oncolytic adenovirus dl1520 and its antineoplastic activity against anaplastic thyroid carcinoma cells. *Endocrinology* 148:5186–5194, 2007
 29. Gestá S, Lolmede K, Daviaud D, et al.: Culture of human adipose tissue explants leads to profound alteration of adipocyte gene expression. *Horm Metab Res* 35:158–163, 2003
 30. DeLany JP, Floyd ZE, Zvonic S, et al.: Proteomic analysis of primary cultures of human adipose-derived stem cells: modulation by adipogenesis. *Mol Cell Proteomics* 4:731–740, 2005
 31. Stolic M, Russell A, Hutley L, et al.: Glucose uptake and insulin action in human adipose tissue: influence of BMI, anatomical depot and body fat distribution. *Int J Obes Relat Metab Disord* 26:17–23, 2002
 32. R Development Core Team: *R: A Language and Environment for Statistical Computing*. Vienna, Austria, R Foundation for Statistical Computing, 2007
 33. Whigham LD, Israel BA, Atkinson RL: Adipogenic potential of multiple human adenoviruses in vivo and in vitro in animals. *Am J Physiol Regul Integr Comp Physiol* 290:R190–R194, 2006
 34. Enan E, Matsumura F: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced changes in glucose transporting activity in guinea pigs, mice, and rats in vivo and in vitro. *J Biochem Toxicol* 9:97–106, 1994
 35. Halleux CM, Servais I, Reul BA, et al.: Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. *J Clin Endocrinol Metab* 83:902–910, 1998
 36. Di Gregorio GB, Yao-Borengasser A, Rasouli N, et al.: Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 54:2305–2313, 2005
 37. Wang ZQ, Cefalu WT, Zhang XH, et al.: Human adenovirus type 36 enhances glucose uptake in diabetic and non-diabetic human skeletal muscle cells independent of insulin signaling. *Diabetes*, 57:1805–1813, 2008
 38. Sakaue H, Ogawa W, Matsumoto M, et al.: Posttranscriptional control of adipocyte differentiation through activation of phosphoinositide 3-kinase. *J Biol Chem* 273:28945–28952, 1998
 39. Tomiyama K, Nakata H, Sasa H, et al.: Wortmannin, a specific phosphatidylinositol 3-kinase inhibitor, inhibits adipocytic differentiation of 3T3-L1 cells. *Biochem Biophys Res Commun* 212:263–269, 1995
 40. Cong L, Chen K, Li J, et al.: Regulation of adiponectin and leptin secretion and expression by insulin through a PI3K-PDE3B dependent mechanism in rat primary adipocytes. *Biochem J* 403:519–525, 2007
 41. Zhang WJ, Wei H, Hagen T, et al.: Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc Natl Acad Sci U S A* 104:4077–4082, 2007
 42. Bandyopadhyay G, Sajan MP, Kanoh Y, Standaert ML, Quon MJ, Lea-Currie R, Sen A, Farese RV: PKC-zeta mediates insulin effects on glucose transport in cultured preadipocyte-derived human adipocytes. *J Clin Endocrinol Metab* 87:716–723, 2002
 43. Li E, Stupack D, Klemke R, et al.: Adenovirus endocytosis via alpha(v) integrins requires phosphoinositide-3-OH kinase. *J Virol* 72:2055–2061, 1998
 44. Li E, Stupack DG, Brown SL, et al.: Association of p130CAS with phosphatidylinositol-3-OH kinase mediates adenovirus cell entry. *J Biol Chem* 275:14729–14735, 2000
 45. Rajala MS, Rajala RV, Astley RA, et al.: Corneal cell survival in adenovirus type 19 infection requires phosphoinositide 3-kinase/Akt activation. *J Virol* 79:12332–12341, 2005
 46. Frese KK, Lee SS, Thomas DL, et al.: Selective PDZ protein-dependent stimulation of phosphatidylinositol 3-kinase by the adenovirus E4-ORF1 oncoprotein. *Oncogene* 22:710–721, 2003
 47. Frese KK, Latorre IJ, Chung SH, et al.: Oncogenic function for the Dlg1 mammalian homolog of the Drosophila discs-large tumor suppressor. *EMBO J* 25:1406–1417, 2006
 48. Tan PH, Xue SA, Manunta M, et al.: Effect of vectors on human endothelial cell signal transduction: implications for cardiovascular gene therapy. *Arterioscler Thromb Vasc Biol* 26:462–467, 2006
 49. Esposito DL, Li Y, Cama A, et al.: Tyr(612) and Tyr(632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells. *Endocrinology* 142:2833–2840, 2001
 50. Orme MH, Alrubaie S, Bradley GL, et al.: Input from Ras is required for maximal PI(3)K signalling in Drosophila. *Nat Cell Biol* 8:1298–1302, 2006
 51. Suire S, Condliffe AM, Ferguson GJ, et al.: Gbetagammias and the Ras binding domain of p110gamma are both important regulators of PI(3)Kgamma signalling in neutrophils. *Nat Cell Biol* 8:1303–1309, 2006
 52. Houseknecht KL, Zhu AX, Gnudi L, et al.: Overexpression of Ha-ras selectively in adipose tissue of transgenic mice: evidence for enhanced sensitivity to insulin. *J Biol Chem* 271:11347–11355, 1996
 53. Tanti JF, Gremaux T, Van Obberghen E, et al.: Insulin receptor substrate 1 is phosphorylated by the serine kinase activity of phosphatidylinositol 3-kinase. *Biochem J* 304:17–21, 1994
 54. Ye J: Role of insulin in the pathogenesis of free fatty acid-induced insulin resistance in skeletal muscle. *Endocr Metab Immune Disord Drug Targets* 7:65–74, 2007
 55. Sakaue M, Bowtell D, Kasuga M: A dominant-negative mutant of mSOS1 inhibits insulin-induced Ras activation and reveals Ras-dependent and -independent insulin signaling pathways. *Mol Cell Biol* 15:379–388, 1995
 56. Mannova P, Beretta L: Activation of the N-Ras-PI3K-Akt-mTOR pathway by hepatitis C virus: control of cell survival and viral replication. *J Virol* 79:8742–8749, 2005
 57. Ehrhardt C, Wolff T, Pleschka S, et al.: Influenza A virus NS1 protein activates the PI3K/Akt pathway to mediate antiapoptotic signaling responses. *J Virol* 81:3058–3067, 2007
 58. Ahmed A, Thompson J, Emilusen L, et al.: A conditionally replicating adenovirus targeted to tumor cells through activated RAS/P-MAPK-selective mRNA stabilization. *Nat Biotechnol* 21:771–777, 2003
 59. Velcich A, Ziff E: Adenovirus E1a ras cooperation activity is separate from its positive and negative transcription regulatory functions. *Mol Cell Biol* 8:2177–2183, 1988
 60. Bardell D: Glucose uptake and lactic acid production of adenovirus type 5-infected HEP-2 cells cultured under exponential growth and stationary phase conditions. *Microbios* 20:139–144, 1977
 61. Atkinson R, Whigham L, Kim Y, et al.: Evaluation of human adenoviruses as an etiology of obesity in chickens. *Am J Clin Nutr* 75:380S, 2002
 62. Xiao J, Chodosh J: JNK regulates MCP-1 expression in adenovirus type 19-infected human corneal fibroblasts. *Invest Ophthalmol Vis Sci* 46:3777–3782, 2005
 63. Alcorn MJ, Booth JL, Coggeshall KM, et al.: Adenovirus type 7 induces interleukin-8 production via activation of extracellular regulated kinase 1/2. *J Virol* 75:6450–6459, 2001
 64. Iacobelli-Martinez M, Nepomuceno RR, Connolly J, et al.: CD46-utilizing adenoviruses inhibit C/EBPbeta-dependent expression of proinflammatory cytokines. *J Virol* 79:11259–11268, 2005
 65. Vilarrasa N, Vendrell J, Sanchez-Santos R, et al.: Effect of weight loss induced by gastric bypass on proinflammatory interleukin-18, soluble tumour necrosis factor-alpha receptors, C-reactive protein and adiponectin in morbidly obese patients. *Clin Endocrinol (Oxf)* 67:679–686, 2007
 66. Engl J, Bobbert T, Ciardi C, et al.: Effects of pronounced weight loss on adiponectin oligomer composition and metabolic parameters. *Obesity (Silver Spring)* 15:1172–1178, 2007
 67. Lin E, Phillips LS, Ziegler TR, et al.: Increases in adiponectin predict improved liver, but not peripheral, insulin sensitivity in severely obese women during weight loss. *Diabetes* 56:735–742, 2007
 68. Jung SH, Park HS, Kim KS, Choi WH, Ahn CW, Kim BT, Kim SM, Lee SY, Ahn SM, Kim YK, Kim HJ, et al.: Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J Nutr Biochem* 19:371–375, 2007
 69. Bougoulia M, Triantos A, Koliakos G: Effect of weight loss with or without orlistat treatment on adipocytokines, inflammation, and oxidative markers in obese women. *Hormones (Athens)* 5:259–269, 2006
 70. Atkinson RL, Dhurandhar NV, Allison DB, et al.: Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes (Lond)* 29:281–286, 2005
 71. Pasarica M, Mashtalir N, McAllister EJ, et al.: Adipogenic human adenovirus Ad-36 induces commitment, differentiation and lipid accumulation in human adipose-derived stem cells. *Stem Cells* 26:969–978, 2008