

Short-Term Overexpression of a Constitutively Active Form of AMP-Activated Protein Kinase in the Liver Leads to Mild Hypoglycemia and Fatty Liver

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AMP-activated protein kinase (AMPK) is a major therapeutic target for the treatment of diabetes. We investigated the effect of a short-term overexpression of AMPK specifically in the liver by adenovirus-mediated transfer of a gene encoding a constitutively active form of AMPK α 2 (AMPK α 2-CA). Hepatic AMPK α 2-CA expression significantly decreased blood glucose levels and gluconeogenic gene expression. Hepatic expression of AMPK α 2-CA in streptozotocin-induced and *ob/ob* diabetic mice abolished hyperglycemia and decreased gluconeogenic gene expression. In normal mouse liver, AMPK α 2-CA considerably decreased the refeeding-induced transcriptional activation of genes encoding proteins involved in glycolysis and lipogenesis and their upstream regulators, SREBP-1 (sterol regulatory element-binding protein-1) and ChREBP (carbohydrate response element-binding protein). This resulted in decreases in hepatic glycogen synthesis and circulating lipid levels. Surprisingly, despite the inhibition of hepatic lipogenesis, expression of AMPK α 2-CA led to fatty liver due to the accumulation of lipids released from adipose tissue. The relative scarcity of glucose due to AMPK α 2-CA expression led to an increase in hepatic fatty acid oxidation and ketone bodies production as an alternative source of energy for peripheral tissues. Thus, short-term AMPK activation in the liver reduces blood glucose levels and results in a switch from glucose to fatty acid utilization to supply energy needs. *Diabetes* 54:1331–1339, 2005

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ACC, acetyl-CoA carboxylase; Ad, adenovirus; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; FFA, free fatty acid; G6Pase, glucose-6-phosphatase; HC, high-carbohydrate/low-fat; LPL, lipoprotein lipase; STZ, streptozotocin; UCP-2, uncoupling protein 2.

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AMP-activated protein kinase (AMPK) is a metabolic master switch mediating adaptation of the cell to variations in nutritional environment (1). Its activity is stimulated by increases in intracellular AMP-to-ATP ratio in response to stresses such as exercise, hypoxia, and glucose deprivation. AMPK has acute effects on energy metabolism pathways and long-term effects involving changes in gene expression. Two antidiabetic adipocyte-secreted hormones, leptin and adiponectin, were recently shown to activate AMPK. Leptin increases fatty acid oxidation in skeletal muscle, both directly and indirectly, via the hypothalamic-sympathetic nervous system axis (2). Adiponectin activates AMPK in skeletal muscle, stimulating glucose utilization, glycogen synthesis, and fatty acid oxidation and, in the liver, increasing fatty acid oxidation and inhibiting glucose production (3). In contrast, the adipocyte-secreted hormone resistin increases glucose production by the liver, probably by inactivating AMPK (4). The discovery that adipocyte-derived hormones have potent metabolic effects is consistent with cross talk occurring between energy store in adipose tissue and organs such as the liver and skeletal muscle. These effects on glucose and lipid metabolism have led to the identification of AMPK as a major pharmacological target for the treatment of metabolic disorders. The antidiabetic agents metformin and thiazolidinediones (5,6) activate AMPK, providing support for the targeting of AMPK in drug development. Studies in animal models of type 2 diabetes have shown that the pharmacological activation of AMPK with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) or metformin decreases blood glucose levels and improves lipid profile (7–9). However, these compounds are not ideal for investigating the beneficial effects of AMPK activation because they may affect AMPK-independent pathways (10). Furthermore, the positive effects of AMPK activation have been shown to be mediated by multiple organs, including skeletal muscle and the liver in particular. The metabolic consequences of AMPK activation, specifically in the liver, and the beneficial effects of this activation on glucose and lipid homeostasis have yet to be reported. We investigated the effects of adenovirus (Ad)-mediated transfer of a gene encoding a constitutively active form of AMPK α 2 (AMPK α 2-CA) in the liver. Expression of AMPK α 2-CA in the liver decreased blood glucose levels and hepatic

gluconeogenic gene expression. The resulting low availability of glucose led to a switch from glucose utilization to fatty acid utilization, associated with a decrease in white adipose tissue mass and lipid accumulation in the liver.

RESEARCH DESIGN AND METHODS

Recombinant Ads. AMPK α 2-CA was created by truncating a full-length myc epitope-tagged rat AMPK α 2 cDNA (a gift from D. Carling, London, U.K.) at residue 312, as described for the AMPK α 1 isoform (11). AMPK α 2-CA was amplified by PCR then subcloned into the pAdTrack shuttle vector, and recombinant adenoviral vector was obtained as previously described (12). Ad expressing β -galactosidase was used as a control. Ads were propagated in AD-293 cells and purified by cesium chloride density centrifugation as previously described (13).

Male C57BL/6j and C57BL/6j *ob/ob* mice (9 weeks old) were maintained under a 12-h light/12-h dark cycle with free access to water and standard mouse diet. Streptozotocin (STZ)-induced diabetic mice were obtained by two intraperitoneal injections of 125 mg/kg STZ 24 h apart. Five days after the first injection, mice with blood glucose levels up to 280 mg/dl were studied further. Animals were killed 48 h postinfection under ketamine/xylazine anesthesia. Tissues were dissected immediately, frozen in liquid nitrogen, and stored at -80°C until analysis. All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals.

Injection of recombinant Ads and fasting/refeeding experiments. Mice were anesthetized with isoflurane, and Ad β -gal or Ad AMPK α 2-CA (1×10^9 plaque-forming units) was injected into the penis vein. After Ad injection, C57BL/6j mice were divided into two groups: fasted and refeed. The fasted group was fed standard diet ad libitum for 24 h then fasted for 24 h. The refeed group had free access to food for 8 h after injection. They were then fasted for 24 h then refeed a high-carbohydrate/low-fat (HC) diet (75% wt/wt sucrose, 15% wt/wt protein, and 3% wt/wt fat) for 16 h. Mice were killed 48 h after Ad injection. Tissue analyses and biochemical analyses of plasma were carried out as previously described (14). Plasma glucagon concentration was assessed by radioimmunoassay (Linco).

Primary culture of hepatocytes and transfection with recombinant Ads. Hepatocytes were isolated from fed male C57BL/6j mice by a modified version of the collagenase method and were transfected with 10 plaque-forming units/cell of Ad β -gal or Ad AMPK α 2-CA (13). Untransfected hepatocytes were treated for 6 h with 500 $\mu\text{mol/l}$ AICAR (Toronto Research Chemicals). For hepatic glucose production, cells were incubated with glucose-free culture medium containing 100 $\mu\text{mol/l}$ $\text{Bt}_2\text{-cAMP}$, 10 mmol/l lactate, and 1 mmol/l pyruvate. We collected culture medium at 4, 8, and 12 h to determine the amount of glucose released. Glucose levels were determined by evaluating the production of NADPH from NADP $^{+}$ in the presence of hexokinase and glucose-6-phosphate dehydrogenase (Roche).

Western blot analysis and AMPK activity. Total protein extracts from hepatocytes and liver samples were obtained as previously described (15). We subjected 50 μg of protein to SDS-PAGE and transferred the resulting bands to nitrocellulose membrane. Blots were probed with antibodies against AMPK α 1, AMPK α 2 (a gift from G. Hardie, Dundee, U.K.), pan-AMPK α , phospho-Thr172-AMPK α , phospho-Ser79-acetyl-CoA carboxylase (ACC) (Cell Signaling), myc tag (clone 9E10; Sigma), and β -galactosidase (Rockland). ACC was detected with streptavidin-horseradish peroxidase (Dako). Total AMPK activity was measured in polyethylene glycol protein precipitate from liver by phosphorylation of the AMARA peptide as previously described (15).

Real-time RT-PCR analysis. Total RNA was isolated with RNA $^{+}$ (Qbiogene), and single-strand cDNA was synthesized from 5 μg of total RNA with random hexamer primers and Superscript II (Invitrogen). Real-time RT-PCRs were carried out with a LightCycler reaction kit (Eurogentec) in a final volume of 20 μl containing 250 ng of reverse-transcribed total RNA, 500 nmol/l of primers (Table 1), 10 μl of $2 \times$ PCR mix, and 0.5 μl of Sybr Green. The reactions were carried out in capillaries in a LightCycler instrument (Roche) with 40 cycles. We determined the relative amounts of the mRNAs studied by means of the second-derivative maximum method, with LightCycler analysis software version 3.5 and 18S RNA as the invariant control for all studies.

Statistical analysis. Results are expressed as means \pm SE. We used Student's *t* test for unpaired data. Differences were considered significant if $P < 0.05$.

RESULTS

AMPK α 2-CA inhibits cAMP-stimulated gluconeogenic gene expression and glucose production in isolated hepatocytes. Cellular AMPK activity was increased by

TABLE 1
Primers used for quantitative real-time PCR

Gene	Accession no.	Forward/reverse primers
GLUT2	X15684	5'-CCTCAAGAGGTAATAATATCCC 5'-CCATCAAGAGGGCTCCAGTC
GK	L38990	5'-CCCTGAGTGGCTTACAGTTC 5'-ACGGATGTGGAGTGTGAAGC
HKII	NM_013820	5'-AACCGAACAAGCTGGTGTAC 5'-TGACACATCTATAGGTGGC
GAPDH	M32599	5'-GTCGGTGTGAACGGATTTGG 5'-GACTCCACGACACTCAGC
L-PK	NM_013631	5'-CTGGAACACCTCTGCCTTCTG 5'-CACAAATTCACCTCCGACTC
PEPCK	NM_011044	5'-GTGCTGGAGTGGATGTTCCGG 5'-CTGGCTGATTCCTGTTCAGG
G6Pase	BC013448	5'-ACTGTGGGCATCAATCTCCTC 5'-CGGGACAGACAGACGTTTCAGC
ACC-1	AF374170	5'-ACAGTGGAGCTAGAATTGGAC 5'-ACTTCCCACAGGACTCTTG
ACC-2	AF290178	5'-TGGGGGAAGATGACAGACTC 5'-GGTTTGGAGTCTGGGGGAGG
FAS	AK080374	5'-AGCGGCCATTTCCATTGCCC 5'-CCATGCCAGAGGGTGGTTG
SCD-1	M21285	5'-CCGGAGACCCTTAGATCGA 5'-TAGCCTGTAAAAGATTTCTGCA AACC
GPAT	NM_008149	5'-CAACACCATCCCCGACATC 5'-GTGACCTTCGATTATGCGATCA
S14	X95279	5'-GTGGTGCAGAACATGGAGCA 5'-TGGGTAAGGATGTGATGGAGG
ChREBP	NM_021455	5'-CTGGGGACCTAACAGGAGC 5'-GAAGCCACCCTATAGCTCCC
SREBP-1	NM_011480	5'-AACGTCACCTCCAGCTAGAC 5'-CCACTAAGGTGCCTACAGAGC
LDLr	NM_010700	5'-GCTCCATAGGCTATCTGCTCTTCA 5'-GCGGTCCAGGGTCACTTTC
HMG-CoA synthase	NM_145942	5'-TGCACGGATCGTGAAGACA 5'-GTCTCTCAGTCACTGTTCTGAACCA
LPL	NM_008509	5'-TGAAGTGGGTTTTCTGAGTAT 5'-GGTTAGCCACCGTTAATATTTG
CPT-1	AF017175	5'-AAGCACCAGCACCTGTACC 5'-CCTTTACAGTGTCCATCCTCTG
MCAD	NM_007382	5'-CGAGCTATGATGAGCCTCTGAA 5'-GTGTCAGAAATCTGATGAAG
AOX	AF006688	5'-AAGAGTTCATTCTCAACAGCCC 5'-CTTGACAGACTCTGAGCTGC
Ketothiolase	NM_146230	5'-TACCGGCAGAACGAGGATGC 5'-TGCTCCATCACTACCTGACTG
HMG-CoA lyase	BC025440	5'-GTCTCTATAGAGGAGAGTTTC 5'-TGGCAGTGGACAGCCAATGC
UCP-2	U69135	5'-GGCCTCTGAAAGGGACTTC 5'-ACCAGCTCAGCAGCTTGACA
18S	X00686	5'-CCATCCAATCGGTAGTAGCG 5'-GTAACCCGTTGAACCCATT

transfection with an adenoviral vector encoding a constitutively active form of AMPK α 2 (Ad AMPK α 2-CA). This protein is a truncated form of AMPK α 2, lacking the auto-inhibitory domain and the region interacting with the regulatory subunits β/γ (Fig. 1A). The infection of primary cultures of mouse hepatocytes with Ad AMPK α 2-CA led to strong expression of myc epitope-tagged AMPK α 2-CA of the expected molecular weight and no change in endogenous AMPK α expression (Fig. 1B). The increase in AMPK activity in hepatocytes resulted in a fourfold increase of ACC phosphorylation (Fig. 1B). The magnitude of this increase is similar to that observed in hepatocytes incubated with AICAR, a known pharmacological activator of AMPK. Incubation of the cells with AICAR increased the amount of the active Thr-172 phosphorylated form of endogenous AMPK. Anti-phospho-AMPK antibodies also recognized the AMPK α 2-CA protein, suggesting that the

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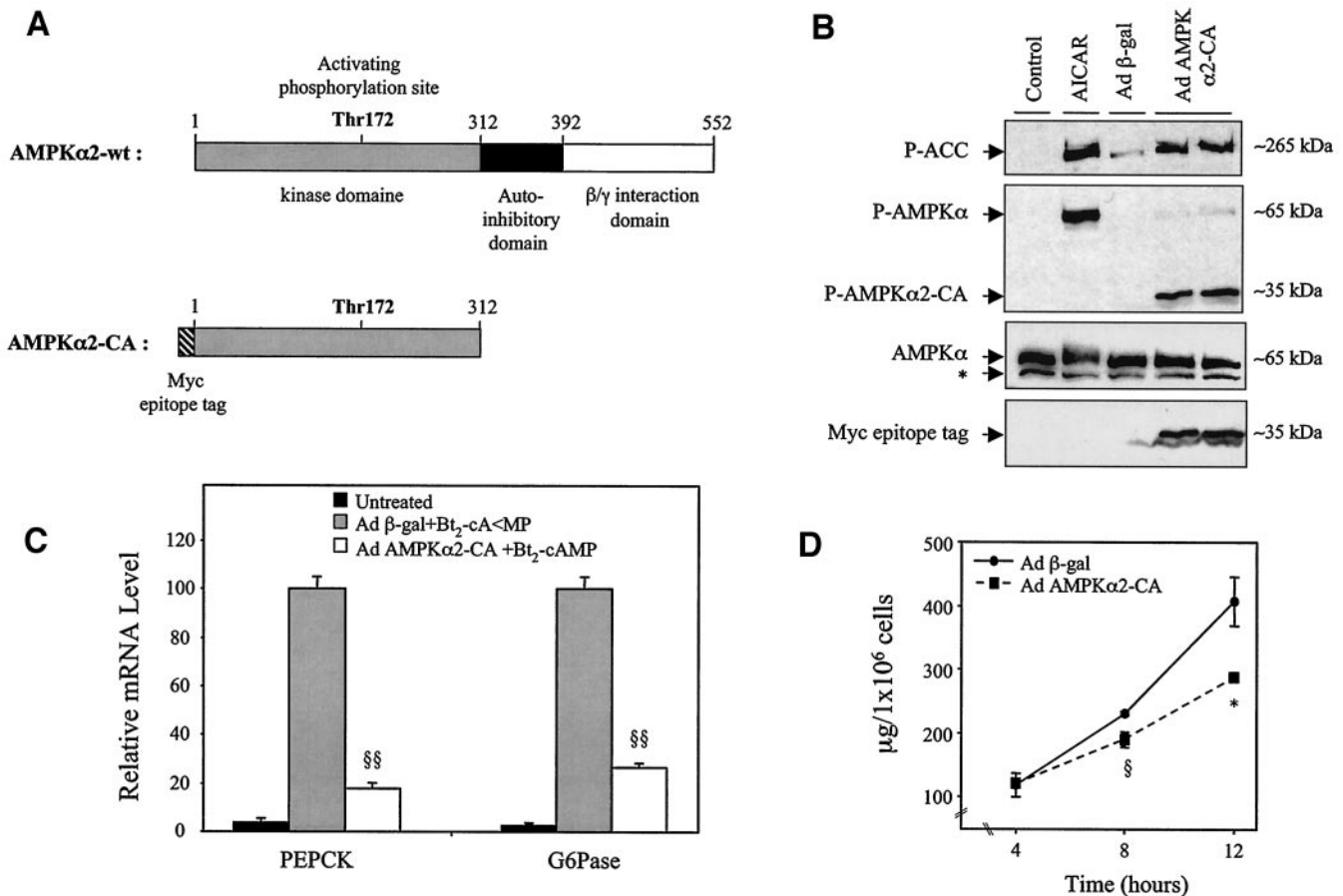


FIG. 1. The expression of AMPK α 2-CA in isolated hepatocytes inhibits cAMP-stimulated gluconeogenic gene expression and glucose production. **A:** Schematic representation of full-length AMPK α 2-wt and AMPK α 2-CA. **B:** Western blot analysis of phospho-ACC, phospho-AMPK α , pan-AMPK α , and myc-tagged AMPK α 2-CA levels in isolated hepatocytes 24 h after transfection with Ad β -gal or Ad AMPK α 2-CA or treated with 500 μ mol/l AICAR for 6 h. *Nonspecific bands. **C:** Expression of the PEPCK and G6Pase genes after transfection with Ad β -gal or Ad AMPK α 2-CA. Each bar represents the mean \pm SE of two separate experiments. §§ P < 0.001 vs. Ad β -gal. **D:** Glucose production in hepatocytes transfected with Ad β -gal (●) or Ad AMPK α 2-CA (■). Each point represents the mean \pm SE of three measures. * P < 0.05, § P < 0.005 vs. Ad β -gal.

truncated AMPK α 2 form is phosphorylated, accounting for its constitutive activity. The pharmacological activation of AMPK has been shown to decrease the level of expression of gluconeogenic genes such as those encoding PEPCK and glucose-6-phosphatase (G6Pase) in hepatoma cells (16,17). The induction of PEPCK and G6Pase gene transcription by Bt₂-cAMP was inhibited by the infection of hepatocytes with Ad/AMPK α 2-CA (Fig. 1C). Consequently, AMPK α 2-CA expression decreased cumulative glucose production by 30% in primary cultures of mouse hepatocytes stimulated for 12 h with Bt₂-cAMP (Fig. 1D). Thus, glucose production in hepatocytes is directly inhibited by activated AMPK.

Expression of a constitutive active form of AMPK α 2 in mice by Ad-mediated gene transfer. The injection of Ad AMPK α 2-CA led to expression exclusively in the liver (Fig. 2A). We checked for the presence of AMPK α 2-CA or β -galactosidase protein in the liver of infected mice by Western blotting (Fig. 2B), and the efficiency of gene transfer was assessed by determining the number of cells positive (>80%) for AMPK α 2-CA and β -gal expression (data not shown). Ad AMPK α 2-CA infection resulted in levels of AMPK activity twice those observed following Ad β -gal infection (Fig. 2C), with no significant change in endogenous AMPK α 1 and α 2 protein levels (Fig. 2B).

Hepatic AMPK α 2-CA expression leads to hypoglycemia and a decrease in PEPCK and G6Pase levels. We investigated the impact of hepatic AMPK α 2-CA on glucose metabolism in vivo by injecting Ad β -gal or Ad AMPK α 2-CA into C57BL/6j mice and determining plasma glucose concentration. Ad AMPK α 2-CA injection triggered a decrease in plasma glucose concentration in the fasted and fed states, 24 and 48 h after infection (Fig. 3A). This effect on blood glucose levels was associated with a physiological response of the pancreatic hormones, a decrease in plasma insulin, and an increase in plasma glucagon concentrations (Table 2). The expression of genes encoding proteins involved in gluconeogenesis, such as PEPCK and G6Pase, in Ad AMPK α 2-CA-infected mice was decreased by nearly twofold relative to Ad β -gal-infected mice (Fig. 3B). Similar experiments were conducted in insulin-resistant *ob/ob* mice. Twenty-four hours after infection with Ad AMPK α 2-CA, blood glucose concentrations had decreased significantly (Fig. 3C), and plasma insulin levels were lower than those in mice infected with Ad β -gal (10,914 \pm 473 pg/ml in Ad β -gal vs. 6,774 \pm 1,112 pg/ml in Ad AMPK α 2-CA, n = 5, P < 0.05). Finally, Ad AMPK α 2-CA was injected into STZ-induced diabetic mice to assess whether AMPK α 2-CA expression was also effective in a murine model of type 1 diabetes. Forty-eight hours after infection with Ad AMPK α 2-CA, blood glucose levels had

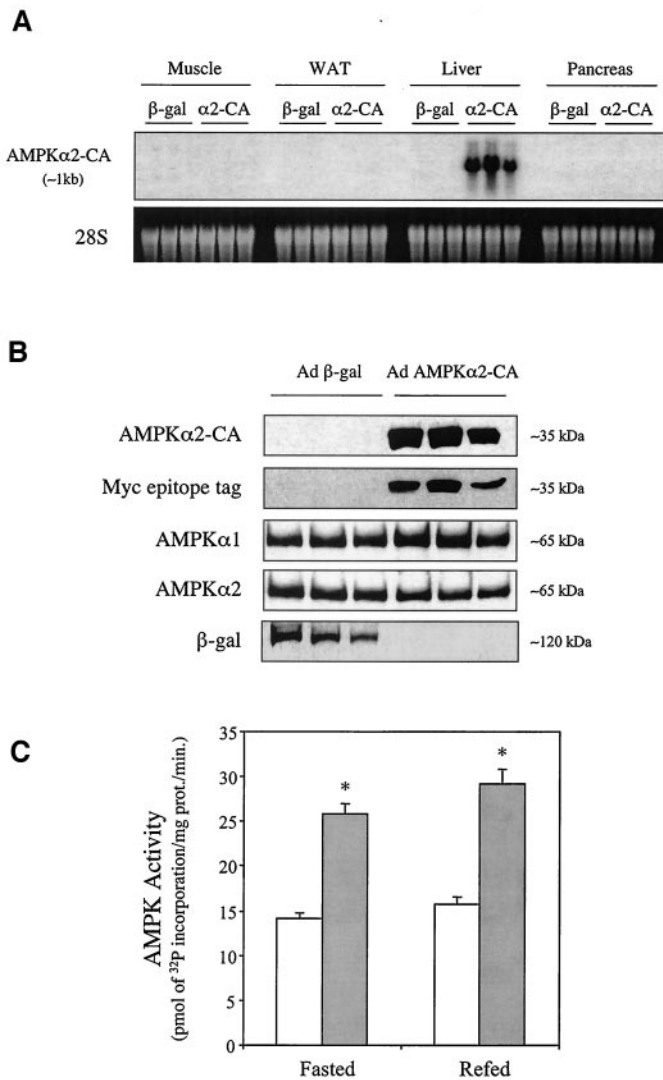


FIG. 2. A: Northern blot analysis of AMPKα2-CA expression in skeletal muscle (vastus lateralis), epididymal fat pad (WAT), liver, and pancreas of mice infected for 48 h with Ad β-gal (β-gal) or Ad AMPKα2-CA (α2-CA). Ethidium bromide-stained 28S RNA was used as a loading control. B: Western blot analysis of AMPKα2-CA, myc-tagged AMPKα2-CA, AMPKα1, AMPKα2, and β-galactosidase levels in the livers of infected mice. C: AMPK activity in the liver of fasted and fed mice 48 h postinfection with Ad β-gal (□) or Ad AMPKα2-CA (■). Each bar represents the mean ± SE of six animals. **P* < 0.05 vs. Ad β-gal.

decreased significantly (Fig. 3E), and plasma insulin levels remained low (42 ± 10 pg/ml in Ad β-gal vs. 41 ± 8 pg/ml in Ad AMPKα2-CA, *n* = 5, NS). Both diabetic *ob/ob* and STZ-treated mice infected with Ad AMPKα2-CA displayed reduced expression levels for the PEPCK and G6Pase genes (Fig. 3D and F). Thus, the activation of AMPK in the liver was sufficient to normalize glucose homeostasis not only in insulin-resistant diabetic mice but also in severe insulinopenic mouse models by reducing the expression of genes encoding the key enzymes of gluconeogenesis.

Hepatic gene expression in normal mice infected with Ad AMPKα2-CA. AMPK has been implicated in the regulation of hepatic gene transcription (15,16,18–20). We therefore thought it likely that AMPKα2-CA expression would modify the pattern of gene expression in the livers of infected mice. The expression of genes encoding proteins involved in glycolysis and lipid synthesis and their

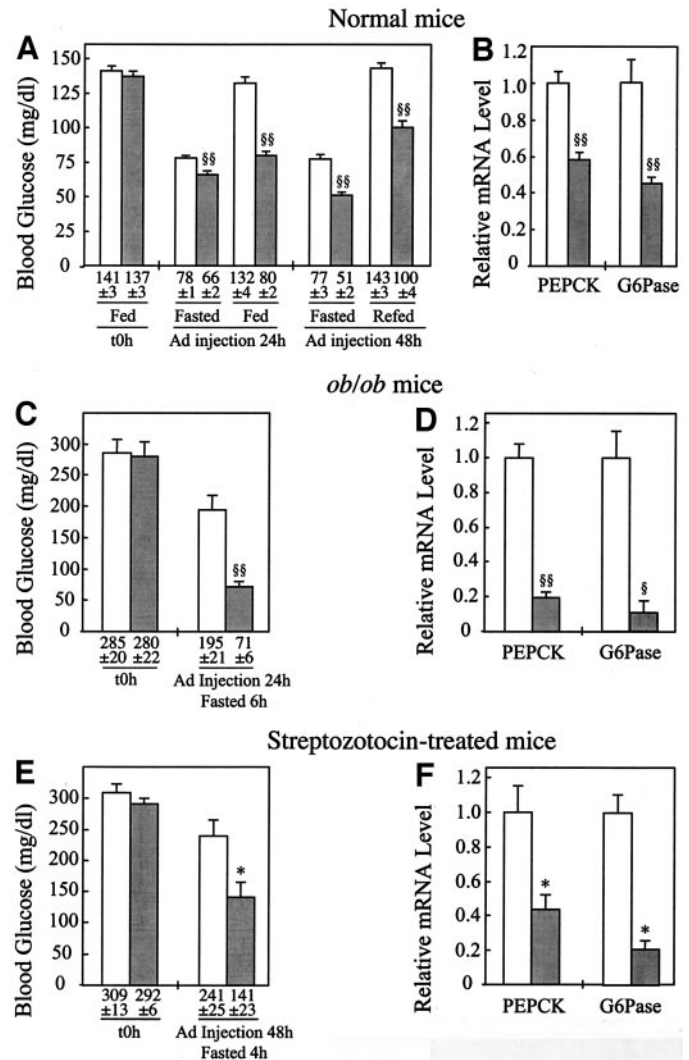


FIG. 3. Effect of hepatic AMPKα2-CA expression on blood glucose levels and liver gluconeogenic gene expression in normal and diabetic mice. A: Blood glucose levels were determined 0, 24, and 48 h after the injection of virus into C57BL/6j mice in the fasted, fed, or fasted/HC refed states (*n* = 12–15). B: Hepatic PEPCK and G6Pase gene expression in mice 48 h after infection, after 24 h of fasting (*n* = 6–8). C: Blood glucose levels in *ob/ob* mice before virus injection in the fed state and 24 h after injection in the 6-h-fasted state (*n* = 5). D: Hepatic PEPCK and G6Pase levels in *ob/ob* mice 48 h after infection in the fed state (*n* = 5). E: Blood glucose levels in STZ-treated mice before virus injection in the fed state and 48 h after injection in the 4-h-fasted state (*n* = 5). F: Hepatic PEPCK and G6Pase gene expression in STZ-treated mice 48 h after infection in the 4-h-fasted state (*n* = 5). Mice were infected with Ad β-gal (□) or Ad AMPKα2-CA (■). Values correspond to blood glucose levels (mg/dl) ± SE. **P* < 0.05, §*P* < 0.005, §§*P* < 0.001 vs. Ad β-gal.

upstream regulators, SREBP-1 (sterol regulatory element-binding protein-1) and ChREBP (carbohydrate response element-binding protein), was induced during the transition between fasting and refeeding in control Ad β-gal-infected mice (Fig. 4). In contrast, the activation of SREBP-1 and ChREBP was considerably reduced in refed AMPKα2-CA-infected mice. This resulted in a lower level of transcriptional activation of downstream target genes, including the genes encoding GLUT2, GK, L-PK, ACC, FAS, S14, and GPAT in response to refeeding (Fig. 4). Protein levels for GK, ACC, and GLUT2 were low, as shown by Western blotting (Fig. 5E and online appendix [available at <http://diabetes.diabetesjournals.org>]). Interestingly, the ex-

TABLE 2
Metabolic parameters in fasted and refed mice 48 h postinfection with Ad β -gal and Ad AMPK α 2-CA

Parameter	Fasted		Refed	
	Ad β -gal	Ad AMPK α 2-CA	Ad β -gal	Ad AMPK α 2-CA
Plasma glucose (mg/dl)	77 \pm 3	51 \pm 2*	143 \pm 3	100 \pm 4*
Plasma insulin (pg/ml)	433 \pm 116	306 \pm 61	1,583 \pm 219	855 \pm 168†
Plasma glucagon (pg/ml)	42 \pm 4	201 \pm 54‡	40 \pm 11	103 \pm 16†
Plasma triglyceride (mmol/l)	1.00 \pm 0.08	0.61 \pm 0.06§	1.22 \pm 0.26	0.32 \pm 0.04*
Plasma FFA (mmol/l)	1.41 \pm 0.07	1.20 \pm 0.09	0.84 \pm 0.18	0.40 \pm 0.04§
Plasma glycerol (μ mol/l)	223 \pm 6	180 \pm 7*	192 \pm 14	128 \pm 16†
Plasma β -hydroxybutyrate (μ mol/l)	480 \pm 65	793 \pm 30*	18 \pm 6	27 \pm 3
Plasma total cholesterol (mmol/l)	1.62 \pm 0.11	1.78 \pm 0.06	1.72 \pm 0.26	1.71 \pm 0.09
Plasma HDL cholesterol (mmol/l)	1.16 \pm 0.13	1.09 \pm 0.06	1.25 \pm 0.19	0.95 \pm 0.09
Liver glycogen (μ g/mg liver wt)	3.9 \pm 0.6	4.2 \pm 1.6	136.3 \pm 8.7	67.5 \pm 27.6*
Liver triglyceride (μ g/mg liver wt)	32 \pm 3	102 \pm 6*	16 \pm 5	50 \pm 5*
Liver cholesterol (μ g/mg liver wt)	3.04 \pm 0.13	3.89 \pm 0.19‡	2.16 \pm 0.11	3.68 \pm 0.21*
Body weight before treatment (g)	24.4 \pm 0.5	25.1 \pm 0.9	24.9 \pm 0.6	25.0 \pm 0.8
Body weight after treatment (g)	22.2 \pm 0.6	21.3 \pm 0.6	22.6 \pm 0.6	20.5 \pm 0.4
Δ of body weight (g)	-2.3 \pm 0.1	-3.9 \pm 0.1†	-2.3 \pm 0.1	-4.5 \pm 0.5*
Liver weight (g)	0.97 \pm 0.02	1.36 \pm 0.03*	1.38 \pm 0.07	1.30 \pm 0.02
Liver weight/body weight (%)	4.4 \pm 0.1	6.4 \pm 0.1*	6.1 \pm 0.3	6.3 \pm 0.1
Epididymal fat weight (g)	0.320 \pm 0.010	0.272 \pm 0.015†	0.273 \pm 0.020	0.251 \pm 0.010
Epididymal fat weight/body weight (%)	1.446 \pm 0.035	1.292 \pm 0.048†	1.218 \pm 0.111	1.219 \pm 0.038

Data are means \pm SE ($n = 6$ –12 animals per group). * $P < 0.001$, † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.005$ vs. Ad β -gal.

pression of SCD-1, a leptin target in the liver (21), was strongly inhibited in both fasted and refed animals (Fig. 4). The genes encoding the LDLr and HMG-CoA synthase, involved in cholesterol uptake and synthesis, were also down-

regulated. Finally, the abundance of HKII and GAPDH mRNAs was higher in fasted and refed animals. The activation of glycolytic and lipogenic genes in response to refeeding was decreased by AMPK α 2-CA and could explain the

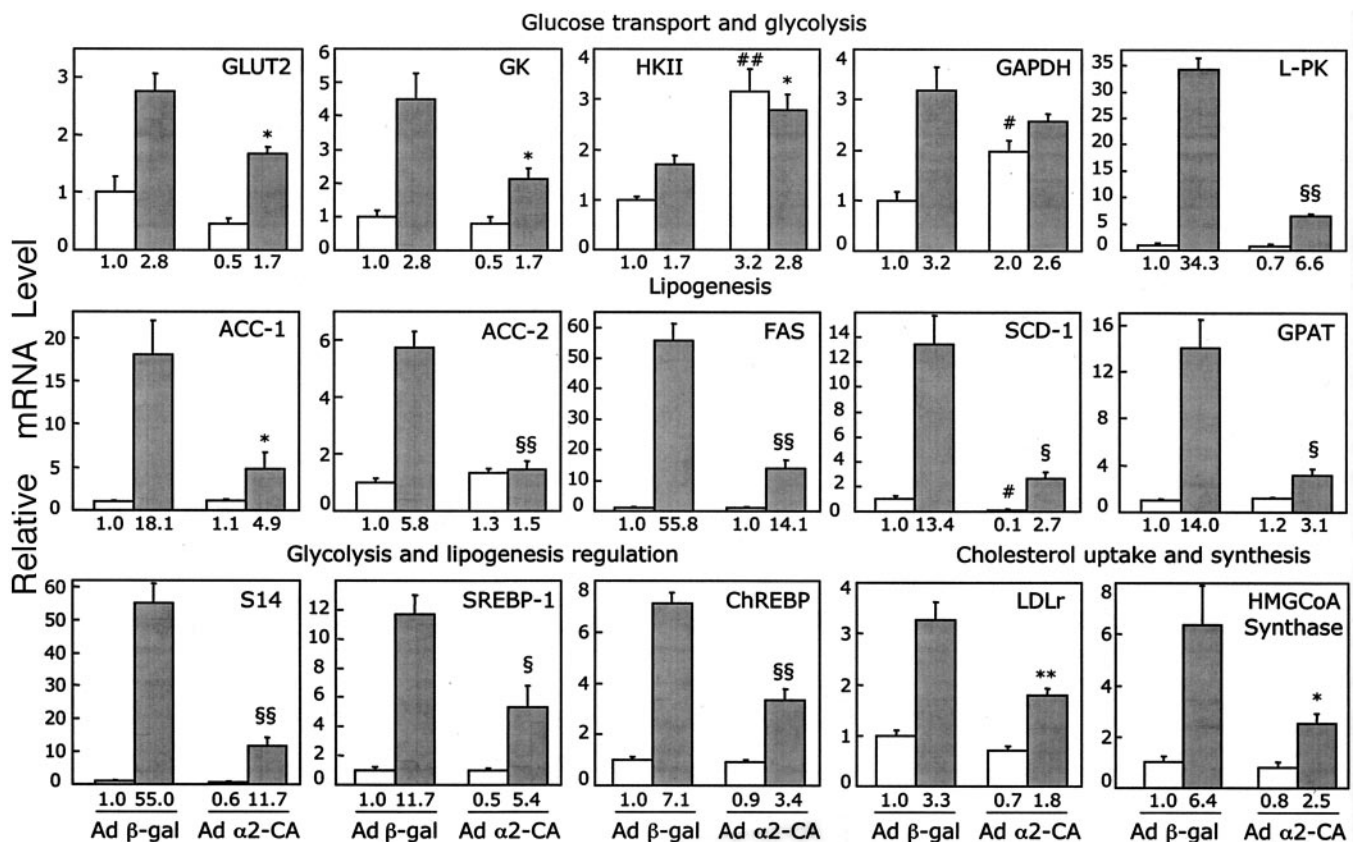


FIG. 4. Effect of hepatic AMPK α 2-CA expression on the expression of genes involved in glucose and lipid metabolism in the livers of fasted and refed normal mice. We injected Ad β -gal or Ad AMPK α 2-CA (α 2-CA) into mice that were fasted (□) or fasted/HF refed (■). Mice were studied 48 h after virus injection and nutritional handle ($n = 3$ –4). Each value indicates the amount of mRNA with respect to that in the fasted mice injected with Ad β -gal, arbitrarily defined as 1. * $P < 0.05$, ** $P < 0.01$, § $P < 0.005$, §§ $P < 0.001$ vs. Ad β -gal refed. # $P < 0.05$, ## $P < 0.01$ vs. Ad β -gal fasted.

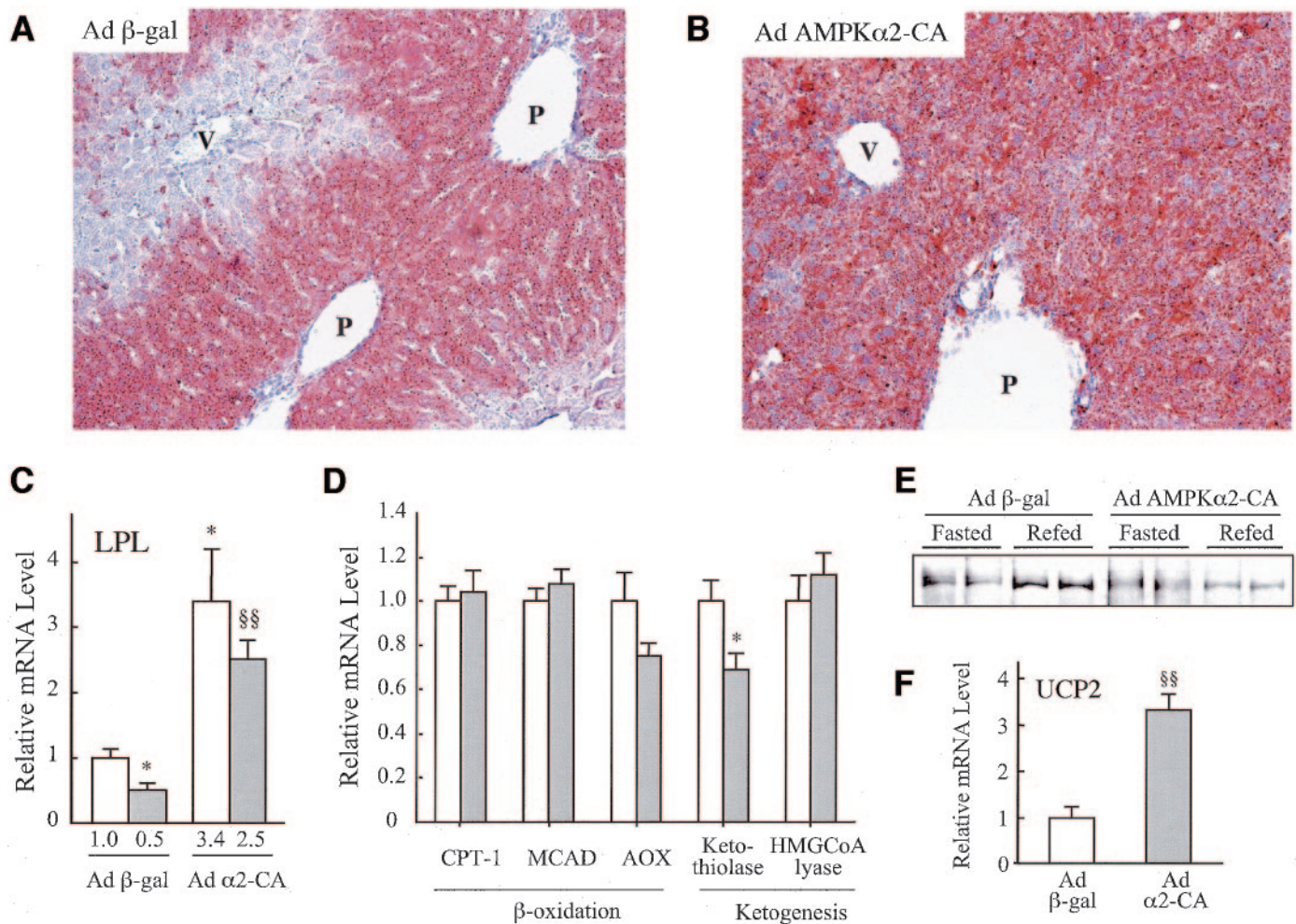


FIG. 5. Effect of hepatic AMPK α 2-CA expression on lipid accumulation in the liver and fatty acids utilization in normal mice. Oil red O–stained liver cryostat sections from 24-h–fasted mice 48 h after the injection of Ad β -gal (A) or Ad AMPK α 2-CA (B). Centrolobular venule (V); portal vein (P). C: LPL gene expression in the livers of mice 48 h after infection with Ad β -gal or Ad AMPK α 2-CA (α 2-CA) in the fasted (\square) or fasted/HC refed (\blacksquare) state ($n = 5$). * $P < 0.05$ vs. Ad β -gal fasted; §§ $P < 0.001$ vs. Ad β -gal refed. Each value indicates the amount of mRNA with respect to that in the fasted mice injected with Ad β -gal, arbitrarily defined as 1. D: Expression of genes encoding proteins involved in β -oxidation and ketogenesis in the livers of 24-h–fasted mice 48 h after the injection of Ad β -gal (\square) or Ad AMPK α 2-CA (\blacksquare) ($n = 4$ –5). * $P < 0.05$ vs. Ad β -gal. E: Western blot analysis of ACC expression in the livers of fasted or refed mice 48 h after infection with Ad β -gal or Ad AMPK α 2-CA. F: UCP-2 gene expression, as described in D. §§ $P < 0.001$ vs. Ad β -gal.

twofold decrease of hepatic glycogen content in the liver of refed mice and the fourfold decrease in plasma triglyceride concentrations (Table 2). In contrast, plasma total cholesterol and HDL cholesterol concentrations were unaffected (Table 2).

Hepatic AMPK α 2-CA expression induces fatty liver in normal mice. Fasting is known to stimulate the release of free fatty acids (FFAs) from adipose tissue, triggering their uptake and oxidation by the liver. The livers of Ad β -gal–infected normal mice contained more triglycerides after 24 h of fasting than during the fed period (Table 2). Lipid deposition, as indicated by oil red O staining, was concentrated in the area around the portal vein (Fig. 5A). The livers of Ad AMPK α 2-CA–infected mice contained three times the amount of triglycerides present in Ad β -gal–infected normal mice (Table 2), with diffuse lipid droplets throughout the liver (Fig. 5B). Liver cholesterol content was also significantly higher in these mice (Table 2). Consistent with this fatty liver, AMPK α 2-CA–infected mice had significantly heavier livers than Ad β -gal–infected normal mice in fasting conditions (Table 2). We investi-

gated this fat accumulation further by monitoring expression of hepatic lipoprotein lipase (LPL). LPL hydrolyzes the triglycerides present in circulating plasma lipoproteins, and the FFAs and monoglycerides released are taken up by tissues for reesterification (for storage) or use as a metabolic fuel. LPL is weakly expressed in the liver but is upregulated in conditions of low carbohydrate availability (22). LPL gene expression was strongly increased by Ad AMPK α 2-CA infection (Fig. 5C), probably accounting for lipid uptake by the liver and the decrease in plasma triglyceride concentration. A significant decrease in epididymal fat pad weight and body weight was observed after Ad AMPK α 2-CA infection (Table 2), probably due to an increase in fat mobilization from the adipose tissue to the liver. This was expected to result in an increase in plasma FFA and glycerol levels in response to the increase in adipose tissue lipolysis. However, plasma FFA and glycerol levels were slightly lower in fasted Ad AMPK α 2-CA–infected animals (Table 2), suggesting that the FFA released by adipose tissue were rapidly utilized by peripheral tissues.

Increase in β -oxidation in livers expressing AMPK α 2-CA in normal mice. We investigated the effect of Ad AMPK α 2-CA infection on lipid utilization in the liver by assessing ketone bodies production, using the surrogate measure of plasma β -hydroxybutyrate concentration. A twofold increase in plasma β -hydroxybutyrate concentration was observed in the fasted state following infection with Ad AMPK α 2-CA (Table 2), suggesting that β -oxidation was activated in these animals. Among the genes encoding proteins involved in fatty acid oxidation and ketogenesis, those encoding CPT-1, MCAD, AOX, ketothiolase, and HMG-CoA lysase showed no change or a slight decrease in expression following AMPK α 2-CA expression (Fig. 5D). The rate-limiting step in the β -oxidation of fatty acids is the transport of fatty acyl-CoA into the mitochondrion by the CPT-1 shuttle system. The key allosteric inhibitor of CPT-1 is malonyl-CoA, which is synthesized from acetyl-CoA by ACC. The levels of ACC protein in the liver of Ad AMPK α 2-CA-infected mice were significantly lower than those in normal mice (Fig. 5E). The resulting decrease in malonyl-CoA levels abolishes inhibition of the rate-limiting transport of fatty acyl-CoA into the mitochondrion for β -oxidation. Finally, levels of uncoupling protein 2 (UCP-2) mRNA were higher in Ad AMPK α 2-CA-infected animals than in normal mice, suggesting that fatty acids may be used to produce heat by uncoupling electron transport from oxidative phosphorylation (Fig. 5F).

DISCUSSION

An important hallmark of diabetes is hyperglycemia, which is associated with an increase in hepatic glucose production. In this study, we showed that short-term hepatic expression of a constitutively active form of AMPK α 2 leads to mild hypoglycemia in normal mice and abolishes hyperglycemia in diabetic *ob/ob* and STZ-induced diabetic mice. These results demonstrate that AMPK activation may have an effect under conditions of severe insulin resistance or insulinopenia and that insulin signaling is therefore not required for this effect. The hypoglycemic effect of AMPK α 2-CA is consistent with the abolition of endogenous glucose production, as suggested by the weaker expression of the gluconeogenic genes PEPCK and G6Pase in AMPK α 2-CA-infected animals. As hepatic glucose production is the primary mechanism regulating glucose flux in the basal state, a decrease in blood glucose levels in the absence of a concomitant rise in basal insulin levels indicates that hepatic AMPK α 2-CA expression probably decreases hepatic glucose production. This correlates with the inhibitory effect of Ad AMPK α 2-CA on glucose output in primary mouse hepatocytes. Moreover, acute exposure to AICAR *in vivo* has been shown to decrease endogenous glucose production (8). Previous reports (7,23–25) have also reported that AICAR decreases blood glucose levels in various rodent models of type 2 diabetes. However, the relative roles played by skeletal muscle and liver in mediation of the hypoglycemic effect of AICAR remain unclear. In muscle-specific AMPK dominant-negative mice, AICAR has a weaker hypoglycemic effect than in control animals (26), suggesting that this compound lowers glycemia at least partly by increasing muscle glucose uptake. Mice with a liver-specific AMPK α knockout (KO) also display an impaired hypoglycemic response to

AICAR, indicating that the liver is involved in the hypoglycemic effect of AICAR *in vivo* (B.V., F.A., unpublished results). The importance of AMPK in the control of glucose output by the liver was recently highlighted by the potent effects of circulating adipocyte-derived hormones on whole-body glucose metabolism. A physiological link has been established between circulating resistin levels and hepatic AMPK activity in the maintenance of blood glucose (4). Short-term adiponectin infusion is known to inhibit both the expression of genes encoding hepatic gluconeogenic enzymes and the rate of endogenous glucose production (27). Similarly, the glucose-lowering effect of metformin has been attributed to a decrease in hepatic glucose production due to a decrease in gluconeogenesis (28). Our results therefore confirm the physiological importance of hepatic AMPK for whole-body glucose homeostasis.

The fatty liver caused by short-term hepatic expression of AMPK α 2-CA was unexpected. Fat accumulation in the liver may be multifactorial, involving an increase in uptake from peripheral fat stores and/or an increase in *de novo* fatty acid synthesis by the hepatocytes. However, *de novo* synthesis is unlikely because lipogenic gene expression was found to be repressed. Lipid uptake from peripheral tissue is demonstrated by the decrease in adipose tissue mass and the upregulation of LPL gene expression. LPL is normally weakly expressed in adult liver and may be induced by limited carbohydrate availability, as occurs during the neonatal period (22). In this situation, LPL expression facilitates the shunting of circulating triglycerides to the liver and the generation of ketone bodies, which can supply energy to peripheral tissues such as the brain and muscle, replacing glucose. Thus, by routing triglycerides to the liver, Ad AMPK α 2-CA-infected mice trigger the mobilization of lipids for energy, thereby decreasing the metabolic stress imposed by the scarcity of glucose. Furthermore, the decrease in FFA levels and the increase in β -hydroxybutyrate levels in the plasma of Ad AMPK α 2-CA-infected mice suggest that the FFA released by the adipose tissue is rapidly used by the liver. Interestingly, Ad AMPK α 2-CA-infected mice had lower fat masses and higher liver weights, reflecting a redistribution of body fat from the adipose tissue to the liver. This finding is consistent with previous reports on long-term AICAR treatment, demonstrating a decrease in abdominal fat mass in normal and obese Zucker rats (25,29). SCD-1 deficiency, which leads to the activation of AMPK, also results in a decrease in adiposity (30). Interestingly, infection with Ad AMPK α 2-CA led to the repression of SCD-1 expression in both fasted and refed conditions. A similar decreased body fat phenotype was also observed in ACC2 KO mice, in which fatty acid oxidation was increased, indicating that high levels of mitochondrial fatty acid oxidation can reduce fat storage in adipose tissue (31). Thus, in Ad AMPK α 2-CA-infected mice, there seems to be a compensatory increase in the use of released FFA for activated β -oxidation. This results in a switch from glucose to fatty acids for energy production. This switch probably involves an increase in basal lipolysis activity due to high plasma concentrations of glucagon and low plasma concentrations of insulin in Ad AMPK α 2-CA-infected animals. AMPK α 2-CA expression upregulated UCP-2 gene expres-

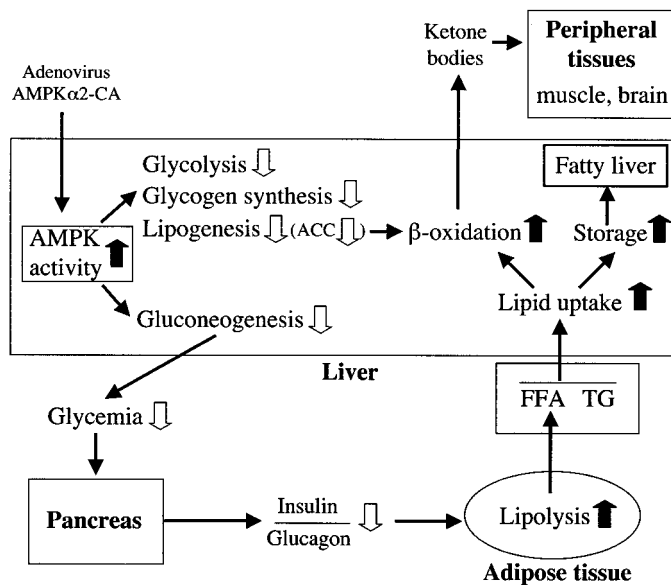


FIG. 6. Model of short-term AMPK α 2 activation in the liver in terms of energy metabolism. Short-term hepatic expression of AMPK α 2-CA results in a metabolic switch from glucose to lipid metabolism. The up or downregulation of metabolic pathways is indicated by arrows (\uparrow for upregulation and \downarrow for downregulation).

sion in the livers of fasted animals. Thus, the UCP-2 protein may be involved in adapting lipid metabolism to an excessive supply of fatty acids for the control of energy status (32). This increase in UCP-2 levels should support higher rates of β -oxidation and may increase fat degradation and the use of fatty acids for energy production. The long-term expression of AMPK α 2-CA may therefore be required to achieve a significant and consistent decline in liver triglyceride content. Whether chronic AMPK α 2-CA expression would lead to an increase in energy expenditure and resistance to diet-induced obesity is now under investigation.

In conclusion, the short-term overexpression of AMPK α 2-CA in the liver results in a metabolic switch from glucose to lipid metabolism (Fig. 6). The lower plasma glucose concentrations in Ad AMPK α 2-CA-infected mice lead to an increase in hepatic lipid utilization, resulting in a decrease in white adipose mass. The concomitant accumulation of hepatic triglycerides leads to the generation of ketone bodies, which are required as alternative substrates to supply energy to peripheral tissues in conditions of low glucose availability. The data reported here indicate that activation of hepatic AMPK is sufficient for controlling hyperglycemia in murine models of diabetes and could lead to decreased adiposity.

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