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Chronic activation of AMP-activated protein kinase-alpha1 in liver leads to decreased adiposity in mice ☆

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ABSTRACT

To assess the metabolic effects of chronic activation of AMP-activated protein kinase (AMPK) in liver, we generated a new transgenic (Tg) mouse model expressing constitutively active (CA)-AMPK-alpha1 in liver. In the short-term activation, the TgCA-AMPK-α1 mice exhibited minimal phenotype, but the Tg liver had elevated sterol regulatory element-binding protein (SREBP)-2 mRNA level and a parallel increase in transcripts of its target genes. UCP2 mRNA level was elevated. In the long-term activation, the TgCA-AMPK-α1 mice had markedly reduced white fat mass. The Tg liver had reduced mRNA expression of SREBP-1c and its target genes. Remarkably, the Tg mice were resistant to a high-fat diet-induced obesity. These data suggest that short-term chronic activation of AMPK-α1 in liver leads to compensatory increase in lipogenic gene expression due to increased SREBP-2 expression, and long-term chronic activation of AMPK-α1 decreases expression of SREBP-1c and its target genes, which results in reduced fat storage.

AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme complex consisting of a catalytic subunit (α) and two regulatory subunits (β and γ) [1]. The α subunit has two isoforms (α1, α2), the β subunit has two isoforms (β1, β2), while the γ subunit has three isoforms (γ1, γ2, and γ3). AMPK activity is suppressed in the absence of AMP due to an autoinhibitory sequence on the α subunit. AMPK acts as a low-energy sensor and is activated by increased AMP:ATP ratio within the cell following a decrease in ATP levels. AMP activates AMPK by several distinctive mechanisms including allosteric activation of AMPK and its upstream kinase AMPKK [2]. Activation of AMPK requires phosphorylation of threonine 172 (T172) in the catalytic (α) subunit by an upstream kinase, the tumor suppressor kinase LKB1 (3). Dephosphorylation of T172 by protein phosphatase 2C (PP2C) *in vivo* leads to inactivation of AMPK [2]. Recently the calmodulin-dependent protein kinase kinase-β (CaMKKβ) and transforming growth factor-beta-activated kinase (TAK1) [3] were identified as alternative upstream kinases of AMPK. Once activated, AMPK switches on ATP-generation pathways, and switches off ATP-utilizing pathways [2]. AMPK is activated by a number of physiologic and pathophysiologic signals such as exercise, hormones, and hypoxia [4]. In addition, metformin and thiazolidinedione (rosiglitazone or pioglitazone), two anti-diabetic drugs, activate AMPK [4].

AMPK plays an important role in regulating hepatic lipid metabolism [5]. In the short-term regulation, AMPK phosphorylates and inactivates acetyl CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, thus inhibiting both cholesterol and fatty acid biosyntheses; at the same time, inactivation of ACC decreases malonyl CoA concentration, which in turn derepresses carnitine palmitoyltransferase (CPT1) and activates fatty acid oxidation [2]. In the long-term, AMPK was thought to regulate hepatic lipogenic gene expression by inhibiting transcription factors [6]. AMPK has been found to downregulate sterol regulatory element-binding protein (SREBP)-1c [7] and inhibit carbohydrate responsive element-binding protein (ChREBP) [8]. The mechanism by which AMPK regulates SREBP-1c expression is not defined. Short-term overexpression of a constitutively active (CA) form of AMPK-α2 in liver decreased lipogenic gene expression by inhibiting expression of SREBP-1c and ChREBP [9]. Somewhat surprisingly, the adenovirus-CA-AMPK-α2-infected mice also had a fatty liver phenotype with a threefold increase in hepatic triglyceride content [9]. The results of short-term overexpression of CA-AMPK-α1 in liver have also been reported [10].

Despite these studies, the consequence of chronic activation of AMPK in liver is not clear. To address this issue, we used a gain-of-function approach to generate a line of transgenic mice selectively expressing CA-AMPK-α1 in liver. Here, we report the findings from the characterization of the CA-AMPK-α1 transgenic mice.

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Materials and methods

General materials. Redivue [α-^{32}P]dCTP (3000 Ci/mmol), [γ-^{32}P]ATP (3000 Ci/mmol), and Hybond nitrocellulose membranes were obtained from Amersham. Peptide HMRSAMSGLHLVKRR (the SAMS peptide) was synthesized by AC Scientific. All DNA oligos were synthesized by Integrated DNA Technologies. Complete mini EDTA-free protease inhibitor cocktail tablets were from Roche. RNA STAT-60 was from TEL-TEST. We obtained anti-AMPK-α and anti-phospho-AMPK-α antibodies from Cell Signaling, anti-AMPK-α1 and anti-AMPK-α2 antibodies from UpState Biotechnology, anti-β-actin antibody from Abcam, and anti-HA tag (Y-11) antibody from Santa Cruz. The secondary antibody horseradish peroxidase-conjugated donkey anti-rabbit or -mouse IgG was from Jackson ImmunoResearch.

Generation of the CA-AMPK-α1 transgenic mice. We generated the constitutively active version of AMPK-α1 truncated at 312 and with the T172D mutation by overlapping PCR from rat liver cDNA according to Stein *et al.* [11] except that the protein has an HA epitope tag (YPYDVPDYA) at the N-terminus. The PCR products were blunted and inserted into the HpaI site of pLiv.11 (a gift from John Taylor, Gladstone Institute, San Francisco) [12]. The 10.5-kb SacII–SpeI transgene fragment was excised from the vector, purified and microinjected into fertilized one-cell eggs of strain B6D2F1/J (The Jackson Laboratory) to produce Tg founders at Mouse Genetic Engineering Facility (MGEF) at the University of Texas at Austin. Tg mice were maintained as hemizygotes by breeding TgCA-AMPK-α1 mice with wild-type (WT) B6D2F1/J mice. All mice were housed in colony cages with a 12-h light/12-h dark cycle and fed a standard chow containing 4.5% (w/w) fat, 24%(w/w) protein and 49.3%(w/w) carbohydrate (Prolab RMH 2500 diet from LabDiet). For animal experiments, non-Tg littermates were used as controls for Tg mice. Mice mostly in the F2 and F3 generations were used for the studies described here. All animal studies were performed with the approval of the Institutional Animal Care and Use Committees at the University of South Alabama and the University of Texas at Austin.

Immunoblotting analysis. Total liver lysate was prepared as described [13]. Proteins (50 μg) were separated by 10% SDS–PAGE and transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in the blocking buffer containing 1× PBS/Tween 20 (PBST, Sigma) and 5% dry milk, incubated with primary antibodies in the blocking buffer for 1 h at room temperature or overnight with gentle shaking, washed three times for 15 min in 1× PBST buffer, and incubated with appropriate secondary antibodies in the blocking buffer for 30 min. The membranes were washed 3 times for 15 min in 1× PBST and immunoreactive proteins were visualized by SuperSignal West Pico Chemiluminescent Substrate (Pierce).

Enzymatic assays of AMPK. AMPK activity was measured by phosphorylation of the SAMS peptide as previously described [14].

Real-time PCR. Real-time PCR was carried out as previously described [15,16]. All reactions were done in triplicate. The relative amounts of mRNAs were calculated using the comparative C_T method (Applied Biosystem 2001, User Bulletin No. 2, Applied Biosystems, Foster City, California). Ribosomal phosphoprotein 36B4 mRNA was used as the invariant control for all studies.

Determination of metabolic parameters. Plasma cholesterol and triglyceride concentrations were measured using the Cholesterol Reagent Set and Triglyceride-GPO Reagent Set (Pointe Scientific). Liver cholesterol and triglyceride concentrations were measured as described previously [17]. Plasma free fatty acids were measured using a NEFA kit (Wako Chemicals). Plasma glucose was measured using the ACCU-CHEK Active glucose meter (Roche). Plasma insulin was measured using a rat insulin ELISA kit (Crystal Chem). Plasma leptin was measured using a mouse leptin ELISA kit (Crystal Chem). Plasma lactate was measured using the Lactate Assay Kit (SUNY at Buffalo). Plasma 3-hydroxybutyrate was measured with Autokit 3-HB R1 Set and R2 Set (Wako Chemicals).

Histology. Liver and adipose tissues from wild-type and transgenic mice were fixed in 10% formalin, processed, and embedded in paraffin blocks. Sections (4–5 μm) were cut and stained with hematoxylin and eosin. The sections were examined and photographed using a Nikon E-600 light microscope (Nikon Instruments Inc.) with digital imaging.

Statistics. Results were expressed as mean ± SE. Statistical analysis was performed using a two-tailed Student's *t* test. The difference between two groups with $P < 0.05$ was considered statistically significant.

Results

Generation of liver-specific CA-AMPK-α1 transgenic mice

Fig. 1A shows the diagram of the Tg vector. The transgene-derived protein CA-AMPK-α1 was detected in livers with anti-AMPK-α antibody that recognized the endogenous AMPK-α1 and -α2 as well as the truncated α1 protein (Fig. 1B). The CA-AMPK-α1 protein was also detected with anti-HA antibody (Fig. 1C). There were no changes in the endogenous AMPK-α1 and α2 protein levels between WT and Tg livers (Fig. 1C). The anti-AMPK-α1 antibody was raised against a peptide corresponding to amino acids 376–392 of AMPK-α1 and did not recognize the truncated CA-AMPK-α1 protein (AA1-312). Fig. 1D shows that Tg mice had a 22% increase in basal AMPK activity in liver compared with WT litter-

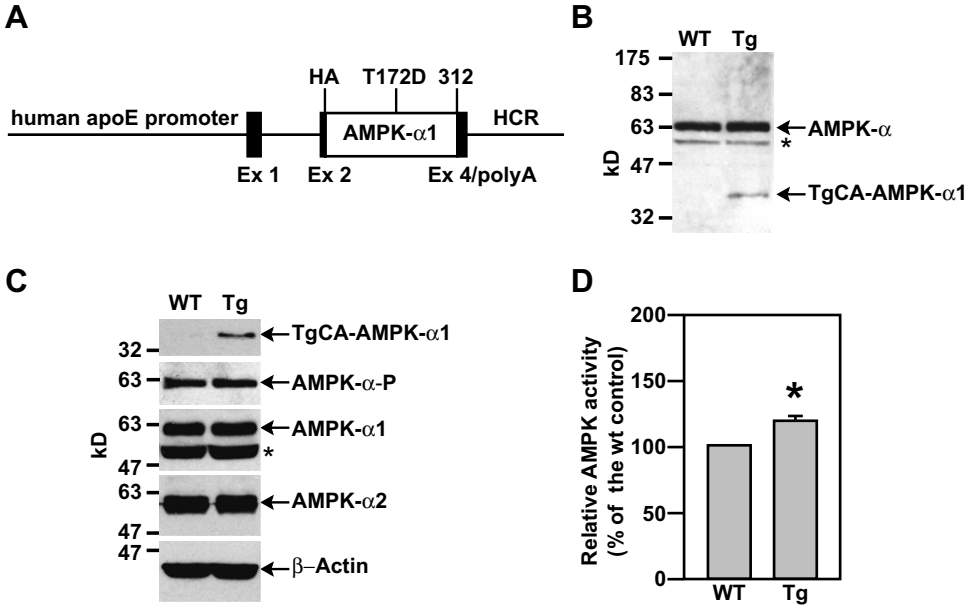

Fig. 1. Generation of CA-AMPK-α1 transgenic mice. (A) Schematic diagram of the transgenic construct containing the human apoE promoter, exon1, intron1 and part of exon2, the HA-tagged AMPK-α1 (AA1 to 312) with T to D mutation at residue 172, exon 4/polyA and the hepatic control region (HCR). (B, C) Analysis of expression of the transgene-encoded protein by immunoblotting. Total liver lysates were prepared from wild-type (WT) and transgenic mouse (Tg) livers. Proteins (50 μg) were separated by 10% SDS–PAGE and transferred onto nitrocellulose membranes. Membranes were blotted with anti-AMPK-α (B), anti-HA, anti-phospho-AMPK-α, anti-AMPK-α1, anti-AMPK-α2, and anti-β actin antibodies (C). The asterisk represents a nonspecific protein band (see Stapleton et al. JBC 271, 611-614, 1996). (D) Quantitative analysis of AMP-activated protein kinase (AMPK) activity in CA-AMPK-α1 transgenic mice. Preparation of total liver lysate and measurement of the enzymatic activity of AMPK in whole liver lysate using the SAMS peptide as the substrate were described in the Materials and methods. The data represent the average of three independent measurements performed in duplicates. $^{*}P < 0.05$.