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Compensatory increase in lipogenic gene expression in adipose tissue of transgenic mice expressing constitutively active AMP-activated protein kinase-alpha1 in liver

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ABSTRACT

We previously described a line of transgenic mice selectively expressing constitutively active AMPK-α1 under the control of liver-specific human apoE promoter with the hepatic control region sequence. In the short-term activation, the CA-AMPK-α1 transgenic mice at age 10–12 weeks exhibited normal hepatic triglyceride content as compared to wild-type mice due to compensatory increase in mRNA expression of genes in the cholesterol and fatty acid synthesis pathways. But it was not known whether the lipogenic gene expression in white adipose tissue also changed. Here we characterized mRNA expression profile of main lipogenic genes in the cholesterol and fatty acid biosynthesis pathway in white adipose tissue. The data show that short-term chronic activation of AMPK in liver caused marked compensatory increase in lipogenic gene expression both in liver due to induction of *Srebp-2* and in white adipose tissue due to upregulation of *Srebp-1c*. These results support the notion that in addition to its well-recognized function for fat storage adipose tissue can play an adaptive role in fatty acid synthesis when fatty acid synthesis is severely reduced in liver, the main lipogenic organ in mammals.

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1. Introduction

AMP-activated protein kinase (AMPK) is a key regulator in hepatic lipid metabolism [1]. 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). Activation of AMPK requires phosphorylation of threonine 172 (T172) in the catalytic (α) subunit by one of the three upstream kinases, liver kinase B1 (LKB1) [2–4], calmodulin-dependent protein kinase kinase (CaMKK)-β [5–7], or transforming growth factor-β (TGFβ)-activated kinase-1 (TAK1) [8].

To further explore the physiological function of AMPK-α1, we used a gain-of-function approach to generate a line of transgenic mice selectively expressing constitutively active (CA)-AMPK-α1 by using the well-characterized liver-specific human apoE promoter with the hepatic control region (HCR) sequence [9]. In the short-term activation, the CA-AMPK-α1 transgenic mice at age 10–12 weeks exhibited minimal phenotype despite the inhibition of hepatic lipid synthesis; the transgenic liver had normal triglyceride content as compared to wild-type mice due to compensatory increase in mRNA expression of genes in the cholesterol and fatty acid synthesis pathways. Plasma triglyceride level was significantly lower in the transgenic mice, but plasma free fatty acid level was unaltered. However, the white fat mass was mildly but not significantly decreased in transgenic mice, suggesting that the white adipose tissue in the transgenic mice had increased fat synthesis in order to maintain the relatively normal fat tissue mass. To test this hypothesis, we characterized mRNA expression profile of main lipogenic genes in the cholesterol and fatty acid biosynthesis pathway in adipose tissue of CA-AMPK-α1 transgenic mice.

2. Methods and materials

Transgenic mice expressing the constitutively active version of AMPK-α1 truncated at 312 and with the T172D mutation under the control of liver-specific human apoE promoter was described previously [9]. Animal experiments were performed with the approval of the Institutional Animal Care and Use Committees at the University of South Alabama. Real-time PCR was performed as previously described [10,11].

3. Results and discussion

3.1. Lipogenic gene expression in Tg CA-AMPK-α1 mice

We previously reported that the CA-AMPK-α1 mice at age 10–12 weeks had markedly increased hepatic lipogenic gene

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Table 1
Relative white fat mRNA levels in wild-type and CA-AMPK-α1 transgenic mice at age 10 to 12 weeks in fed and fast states.

mRNA	WT		Transgenic	
	Fed	Fast	Fed	Fast
SREBP pathway				
SREBP-1a	1	0.35	1.41	0.35
SREBP-1c	1	0.34	4.89	0.18
SREBP-2	1	0.54	0.63	0.19
SCAP	1	0.44	0.93	0.73
Insig-1	1	0.71	9.65	0.46
Insig-2b	1	0.72	1.83	2.25
Fatty acid and cholesterol synthesis				
ACL	1	0.56	8.0	0.14
ACC	1	0.27	16.68	0.8
FAS	1	0.16	19.16	0.21
SCD-1	1	0.16	2.19	2.41
LCE	1	0.81	4.11	0.13
ME	1	0.46	14.52	1.15
HMG CoA synthase	1	0.81	3.63	1.52
HMG CoA reductase	1	0.62	0.44	0.19
Insulin signaling and others				
Insulin receptor	1	0.76	1.58	2.0
IRS-1	1	0.35	2.41	1.79
IRS-2	1	0.54	1.96	1.07
IRS-3	1	0.28	1.78	1.21
PEPCK	1	1.32	1.23	4.2
Glut 1	1	1.29	0.47	0.35
Glut 4	1	0.12	10.34	0.35

Total RNAs from white fat tissues of wild-type and transgenic mice (n = 5–6) at age 10–12 weeks were prepared with RNA STAT-60, treated with DNase I (Ambion), reverse transcribed by using the First-Strand cDNA Synthesis System (Invitrogen), and quantified by real-time PCR as previously described [10,11]. All reactions were done in triplicate. The relative amount of all mRNAs was calculated using the comparative C_T method (Applied Biosystem 2001, User Bulletin No. 2, Applied Biosystems, Foster City, California). The mRNA level of each gene of wild-type mice was arbitrarily set as 1. Each value represents the average of three measurements. The ribosomal phosphoprotein 36B4 was used as the invariant control. Gene-specific primers were described previously [10,11,16,18] ACC, acetyl CoA carboxylase; ACL, ATP citrate lyase; FAS, fatty acid synthase; HMG CoA synthase, 3-hydroxy-3-methylglutaryl CoA synthase; HMG CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; Insig, insulin-induced gene; IRS, insulin receptor substrate; LCE, long-chain fatty acyl elongase; ME, malic enzyme; PEPCK, phosphoenolpyruvate carboxykinase; SCD-1, stearoyl CoA desaturase-1; SREBP, sterol responsive element-binding protein; SCAP, SREBP-cleavage activating protein.

expression [9]. To determine whether CA-AMPK-α1 transgenic mice had altered lipogenic gene expression in the white fat tissue in fed state, we measured mRNA levels of lipogenic genes in the white fat tissue of wild-type and transgenic mice by real-time PCR. Table 1 shows the dramatic changes of mRNA levels in adipose tissue of the transgenic mice. Notably, SREBP-1c mRNA level rose 4.9-fold and SREBP-1a mRNA level rose 41%, whereas SREBP-2 mRNA levels fell 37% in the epididymal fat tissue of the transgenic mice. As a result, all the mRNA levels of SREBP-1c target genes increased. Insig-1 mRNA level rose 9.6-fold, ACL mRNA level 8-fold, ACC mRNA level 16.8-fold, FAS mRNA 19.2-fold and ME mRNA level 14.5-fold. The mRNA levels of other lipogenic genes (SCD-1 and LCE) increased 2- to 4-fold. Similar to SREBP-2, HMG CoA reductase mRNA level fell 54%. SREBP-2 was shown to be more specific for the control of cholesterologenic genes such as HMG CoA reductase and HMG-CoA synthase [12]. Somewhat surprisingly, HMG CoA synthase mRNA level increased 3.6-fold. The increase of PEPCK mRNA level was minimal. All the mRNA levels of the genes (insulin receptor, insulin receptor substrate or IRS-1, -2 and -3) in the insulin signaling pathway rose 1.5- to 2.5-fold. Remarkably, the mRNA level of the major insulin-regulated glucose transporter Glut-4 in adipocytes soared 10.3-fold, whereas the basal glucose transporter Glut-1 mRNA was not increased, indicating dramatically enhanced insulin sensitivity in the adipose tissue of transgenic mice.

3.2. Fasting response in CA-AMPK-α1 transgenic mice

If the dramatic elevation of lipogenic mRNAs in white adipose tissue of CA-AMPK-α1 transgenic mice is caused by increased insulin sensitivity, those lipogenic mRNA levels should decline markedly when the CA-AMPK-α1 transgenic mice are fasted and insulin levels drop. To test this hypothesis, we analyzed mRNA levels in adipose tissue of wild-type and transgenic mice after a 12-h fast. The data are presented in Table 1 and illustrated in Fig. 1A. The mRNA level of SREBP-1c, a direct target of insulin action [13], declined dramatically after 12-h fasting of the transgenic mice. The mRNA for SREBP-1a, which activates both cholesterol and fatty acid synthesis [12], declined by 75%. The mRNA level of SREBP-2 was decreased by 70%. All the SREBP-1c target mRNAs declined. These include mRNAs for Insig-1, a regulator of the proteolytic processing and maturation of SREBPs [14]; ATP-citrate lyase, which catalyzes the formation of acetyl CoA, the precursor for fatty acid synthesis, from citrate; two major enzymes acetyl CoA carboxylase and fatty acid synthase in the fatty acid synthesis pathway; long-chain fatty acyl elongase, a SREBP-target gene that extends fatty acid chain length [15]; malic enzyme, which provides substrate NADPH for fatty acid synthesis. The mRNA of SCD-1, a gene that is controlled by both SREBPs and liver X receptor (LXR), did not change significantly [10]. The mRNA levels for HMG CoA synthase and HMG CoA reductase, two key enzymes in cholesterol synthesis, also declined, presumably due to the fall of SREBP-2 mRNA. The mRNA level for insulin receptor in insulin signaling was not significantly changed, but IRS-1, IRS-2 and IRS-3 mRNA levels declined. The mRNA for Glut-4 increased dramatically in non-fasted transgenic mice, and its mRNA declined to normal when mice were fasted. The mRNA for PEPCK, another insulin-regulated gene, rose to even a greater extent in transgenic mice than in wild-type mice upon fasting. Similar fasting responses in lipogenic genes expression were observed in transgenic liver (Fig. 1B). Altogether, these data are consistent with the notion that hepatic activation of AMPK-α1 in transgenic mice increased insulin sensitivity in adipose tissue as well as in liver, leading to dramatic elevation of lipogenic gene expression.

Kuriyama et al. (2005) reported similar compensatory increase in lipogenic gene expression in adipose tissue in mice with conditional deficiency of SREBP-cleavage activating protein (SCAP) in liver [16]. Mice with liver-specific deletion of *Scap* gene have profound reduction in proteolysis of all three SREBPs, resulting in marked reduction of hepatic lipogenic gene expression and lipid synthesis [17], which leads to compensatory increases in fatty acid synthesis in non-hepatic tissues, mainly white adipose tissue [16]. Here expression of constitutively active AMPK-a1 in liver inhibits hepatic lipid synthesis by inhibiting HMG CoA reductase and acetyl CoA carboxylase; this led to compensatory increase in lipogenic gene expression both in white adipose tissue due to 4.9-fold upregulation of *Srebp*-1c (Fig. 1A) and in liver due to 1.6-fold induction of *Srebp*-2 (Fig. 1B).

In summary, the data in this paper demonstrate that short-term chronic activation of AMPK in liver caused compensatory increase in lipogenic gene expression not only in liver but also in white adipose tissue. Our results support the notion that in addition to its well-recognized fat storage function adipose tissue can play an adaptive role in fat acid synthesis when fatty acid synthesis is severely reduced in liver, the main lipogenic organ in mammals.

4. Conflict of interests

None declared.

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