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# Role of Krüppel-like factor 15 in PEPCK gene expression in the liver

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**Abstract**

Regulation of hepatic gene expression is important for energy homeostasis. We now show that hepatic expression of the gene for the transcription factor Krüppel-like factor 15 (KLF15) is increased by food deprivation and reduced by feeding in mice. Expression of the KLF15 gene in mouse liver was also down-regulated by a euglycemic–hyperinsulinemic clamp and was increased by inhibition of phosphatidylinositol 3-kinase. In cultured rat hepatocytes, KLF15 gene expression was induced by dexamethasone and a non-hydrolyzing analog of cAMP, and this effect was inhibited by insulin in a manner dependent on phosphatidylinositol 3-kinase signaling. Forced expression of KLF15 in cultured hepatocytes increased both the expression and the promoter activity of the gene for phosphoenolpyruvate carboxykinase (PEPCK). These results suggest that insulin and its counteracting hormones regulate the hepatic expression of KLF15, and that this transcription factor contributes to the regulation of PEPCK gene expression in the liver.


*Keywords:* Gene expression; Insulin action; Liver; KLF15; PI 3-kinase

The liver plays a prominent role in fuel metabolism. It is both the only glycogen reservoir from which free glucose can be released into the circulation and is the major site for gluconeogenesis [1]. It is also the major site of the synthesis and oxidation of fatty acids as well as a source of secreted triglycerides [1]. Fuel metabolism in the liver is dependent on the regulation of gene expression both by insulin and by hormones that counteract the effects of insulin. The expression of genes whose products contribute to fatty acid synthesis is up- or down-regulated by insulin and glucagon, respectively [2,3]. Genes for gluconeogenic enzymes, such as those for phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), are induced by glucagon and glucocorticoids, and these effects are inhibited by insulin [1,2]. Insulin and glucagon are major humoral regulators of the expression of PGC-1α [4–6], a transcriptional coactivator that participates in the regulation of both gluconeogenesis and fatty acid oxidation [4,6,7].

Although these various observations indicate the importance of hormonal regulation of hepatic gene expression in fuel homeostasis, only a small number of transcription factors that are directly regulated by insulin and its counteracting hormones have been identified. The hepatic abundance of sterol regulatory element-binding protein 1c (SREBP1c), which contributes to fatty acid synthesis, is increased by insulin [3]. In addition, the transactivation activities of FoxO1 and cAMP-responsive element-binding protein (CREB) are

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controlled by their phosphorylation status, which is modulated by insulin and glucagon, respectively [8,9].

Krüppel-like factor (KLF) 15, a member of the KLF family of transcription factors, was first identified as a protein that binds to the promoter of the gene for CLC-K1, a kidney-specific CLC chloride channel [10]. KLF15 was subsequently shown to regulate the genes for the glucose transporter GLUT4 in adipocytes [11] and for acetyl-CoA synthetase 2 in muscle cells [12]. Given that, among the major organs, the KLF15 gene is expressed at the highest level in the liver [10], we investigated whether KLF15 contributes to the regulation of fuel metabolism in the liver. We now show that hepatic expression of the KLF15 gene is altered in response to nutritional and hormonal changes, and that KLF15 is able to increase transcription of the PEPCK gene in hepatocytes.

## Materials and methods

*Animals and adenoviral vectors.* Animal procedures were performed in accordance with the guidelines of the Animal Ethics Committee of Kobe University Graduate School of Medicine. Adenoviral vectors encoding dominant negative (AxCAΔp85) [13] or constitutively active (AxCAMyr-p110) [14] forms of phosphatidylinositol 3-kinase (PI 3-K) were described previously. A rat cDNA encoding KLF15 was isolated by the polymerase chain reaction (PCR), and an adenoviral vector containing this cDNA (AxCAKLF15) was generated with the use of an Adenovirus Expression Kit (Takara, Tokyo, Japan) as described previously [14]. For analysis of gene expression in mouse liver, 8–9-week-old male C57BL/6 mice were killed either in the randomly fed state, after food deprivation for 24 h, or after refeeding for various times after food deprivation for 24 h. The liver was removed and total RNA was extracted. To express the dominant negative form of PI 3-K in the liver, we injected $5 \times 10^8$ plaque-forming units (PFU) of AxCAΔp85 or of a control virus encoding β-galactosidase (AxCALacZ ) [13] into the tail vein of 10-week-old male BALB/c mice. The mice were killed in the randomly fed state 3 days after injection; the insulin-induced association of PI 3-K activity with IRS proteins in the liver was almost completely inhibited by injection of AxCAΔp85, as previously described [15]. For euglycemic–hyperinsulinemic clamp analysis, human regular insulin was infused into male C57BL/6 mice (8–10 weeks old) at a rate of 2.5 mU/kg of body mass per minute for 120 min and 40% glucose was infused with a variable-rate infusion pump essentially as described [16]. Blood samples were collected from the tip of the tail every 10 min for measurement of the glucose concentration, which was clamped at 100–120 mg/dl. In control animals, saline was infused instead of insulin and glucose. After 120 min, the mice were killed for analysis of hepatic gene expression.

*Northern blot analysis, real-time quantitative PCR, assay of PEPCK promoter activity, immunoblot analysis, and primary culture and infection of hepatocytes.* For Northern blot analysis, probes for PEPCK and G6Pase mRNAs were as described [17] and a $^{32}$P-labeled full-length mouse KLF15 cDNA, isolated with the use of PCR, was used as a probe for KLF15 mRNA. The autoradiograms of Northern blots were visualized and the signal intensity was quantitated with a BAS2500 image analyzer (Fujifilm, Tokyo, Japan). Rat hepatocytes were isolated, cultured, and subjected to adenoviral infection as described [17]. For analysis of gene expression, the cultured hepatocytes (infected or not) were incubated in the absence or presence of 500 nM dexamethasone (Dex), 100 μM 8CPT-cAMP, 100 nM insulin, or 30 μM LY294002 for 9 h unless indicated otherwise. For real-time quantitative PCR analysis, cDNA synthesized from total RNA was analyzed in a Sequence Detector (model 7900, Applied Biosystems) with specific primers and SYBR Green PCR Master (Perkin–Elmer Life Sciences). The relative abundance of mRNAs was calculated with 36B4 mRNA as the invariant control. The primers used were as follows: mouse KLF15, 5′-CCCAATGCCGCCAAACCTAT-3′ (sense) and 5′-GAG GTGGCTGCTCTTGGTGTACATC-3′ (antisense); mouse PEPCK, 5′-GAGATAGCGGCACAAT-3′ (sense) and 5′-TTCAGAGACT ATGCGGTG-3′ (antisense). Mouse 36B4 primers were as described previously [16,18]. The activity of the PEPCK gene promoter was determined as described previously [19] with the use of HL1C cells (kindly provided by D.K. Granner, Vanderbilt University), which contain a PEPCK gene promoter sequence fused to the chloramphenicol acetyl transferase (CAT) gene [20]. Polyclonal antibodies to KLF15 were generated by immunizing rabbits with a keyhole limpet hemocyanin-conjugated synthetic peptide corresponding to the COOH-terminal region of rat or mouse KLF15 (CHRFPRSSRAV RAIN). The nuclear fraction (~50 μg of protein) prepared with a Nuclear/Cytosol Fractionation Kit (BioVision Research Products, Mountain View, CA) was subjected to immunoblot analysis with the polyclonal antibodies to KLF15.

## Results

### *Role of insulin in KLF15 gene expression in mouse liver*

Northern blot analysis revealed that, among the major organs, the abundance of KLF15 mRNA was greatest in the liver of mice in the randomly fed state (Fig. 1A), consistent with previous observations [10]. The amount of KLF15 mRNA in the liver of mice increased about twofold in response to food deprivation for 24 h and decreased to levels below that apparent in the randomly fed state in response to subsequent refeeding for 6 or 12 h (Fig. 1B). The abundance of PEPCK mRNA showed changes similar to those apparent for KLF15 mRNA during the fasting–refeeding cycle.

After feeding, the liver is exposed to high levels not only of insulin but also of glucose. To investigate exclusively the role of insulin in expression of the KLF15 gene in vivo, we performed euglycemic-hyperinsulinemic clamp analysis. The circulating glucose concentration was thus clamped at ~100–120 mg/dl while insulin was continuously infused at a rate of 2.5 mU/kg per minute. Reverse transcription (RT) and quantitative real-time PCR analysis revealed that the hepatic abundance of both KLF15 and PEPCK mRNAs was decreased by the clamp procedure (Fig. 1C), suggesting that insulin inhibits expression of the KLF15 gene in the liver.

PI 3-K plays a key role in the metabolic effects of insulin [21]. We have previously shown that forced expression of a dominant negative form of PI 3-K (Δp85) with the use of an adenoviral vector blocked a variety of biological activities of insulin in mouse liver [15]. In the randomly fed state, mice injected with this vector showed a greater abundance of KLF15 mRNA in the