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Role of Hepatic STAT3 in the Regulation of Lipid Metabolism

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Regulation of hepatic gene expression is largely responsible for the control of nutrient metabolism. We previously showed that the transcription factor STAT3 regulates glucose homeostasis by suppressing the expression of gluconeogenic genes in the liver. However, the role of STAT3 in the control of lipid metabolism has remained unknown. We have now investigated the effects of hepatic overexpression of STAT3, achieved by adenovirus-mediated gene transfer, on glucose and lipid metabolism in insulin-resistant diabetic mice. Forced expression of STAT3 reduced blood glucose and plasma insulin concentrations as well as the hepatic abundance of mRNA for phosphoenolpyruvate carboxykinase. However, it also increased the plasma levels of triglyceride and total cholesterol without affecting those of low density lipoprotein- or high density lipoprotein-cholesterol. The hepatic abundance of mRNAs for fatty acid synthase and acetyl-CoA carboxylase, both of which catalyze the synthesis of fatty acids, was increased by overexpression of STAT3, whereas that of mRNAs for sterol regulatory element-binding proteins 1a, 1c, or 2 was unaffected. Moreover, the amount of mRNA for acyl-CoA oxidase, which contributes to β -oxidation, was decreased by forced expression of STAT3. These results indicate that forced activation of STAT3 signaling in the liver of insulin-resistant diabetic mice increased the circulating levels of atherogenic lipids through changes in the hepatic expression of genes involved in lipid metabolism. Furthermore, these alterations in hepatic gene expression likely occurred through a mechanism independent of sterol regulatory element-binding proteins.

The liver plays a key role in the regulation of nutrient metabolism in living animals. Such regulation is achieved by changes in the activity or abundance of enzymes that function in glucose or lipid metabolism (11, 14). Various transcription factors participate in control of the genes for such enzymes and thereby contribute to the regulation of nutrient metabolism. For example, forkhead transcription factor O1 (FOXO1) and cAMP-responsive element-binding protein are essential for induction of the genes for gluconeogenic enzymes

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(8, 9, 13), whereas sterol regulatory element-binding proteins (SREBP1a, SREBP1c, SREBP2) play a central role in regulation of the genes for various lipogenic enzymes (2, 16).

We recently showed that mice lacking signal transducer and activator of transcription 3 (STAT3) specifically in the liver manifest increased glucose production and expression of gluconeogenic genes in the liver, indicating that STAT3 is a physiological regulator of hepatic gluconeogenesis (4). Moreover, forced expression of an active form of STAT3 in the liver suppressed hepatic glucose production and markedly ameliorated glucose intolerance in insulin-resistant diabetic animals (4), suggesting that STAT3 signaling in the liver is a potential therapeutic target in diabetes mellitus.

Glucose metabolism and lipid metabolism in the liver are closely related, such that individuals with diabetes mellitus frequently manifest dysregulation of lipid metabolism (17). Evaluation of the clinical utility of modulation of hepatic STAT3 signaling thus requires characterization of the effects of activation of such signaling on lipid metabolism. Moreover, whereas in our previous study we used a self-dimerized, oncogenic mutant of STAT3 (1) to activate STAT3 signaling in the liver (4), it has remained unclear whether wild-type STAT3 might also have the ability to ameliorate glucose intolerance in diabetic animals. In the present study, we have therefore investigated whether liver-specific expression of wild-type STAT3 exerts beneficial effects on both glucose and lipid metabolism in insulin-resistant diabetic animals.

MATERIALS AND METHODS

Mice and adenoviral vectors

The study was approved by the Animal Experimentation Committee of Kobe University. Adenovirus vectors encoding wild-type mouse STAT3 (AxCASTAT3) (4) or β -galactosidase (AxCALacZ) (10) were described previously. Eight-week-old male *Lepr^{-/-}* mice (C57BL/KsJ-db/db; Clea Japan), which lack functional leptin receptors and manifest insulin-resistant diabetes (6), were injected through the tail vein with the adenovirus vectors at the indicated number of plaque-forming units (PFU). Various metabolic parameters and hepatic gene expression were assayed with mice in the randomly fed state 4 days after adenovirus injection. Metabolic parameters including concentrations of blood glucose, plasma insulin, and plasma lipids were assayed as described (10, 12).

Hepatic gene expression

Hepatic gene expression was evaluated by reverse transcription and real-time polymerase chain reaction analysis with 36B4 mRNA as the invariant control, as described (4). The primers used were as follows: STAT3, 5'-CCAACAGCCGCCGTAGTGAC-3' and 5'-TGGCTCTTGAGGGTTTTGTAGTTGA-3'; FOXO1, 5'-TAAGGGCGACAGCAACAGCTC-3' and 5'-CTGCACTCGAATAAACTTGCTGTGA-3'; SREBP2, 5'-GCCAGCCCTACCCGTACACA-3' and 5'-CGCCAGCTTGACAAT-3'; acetyl-CoA carboxylase (ACC), 5'-GGATGACAGGCTTGACGCTATG-3' and 5'-GGAACGTAAGTCGCCGGATG-3'; 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), 5'-CGGAAGCTATGGTTGACGTA-3' and 5'-GGCCACATGCGATGTAGAT-3'; acyl-CoA oxidase (ACO), 5'-TGACCTGCCGAGCCAGCGTAT-3' and 5'-GACAGAAGTCAAGTTCCACGCCACT-3'; and carnitine palmitoyltransferase 1 (CPT1), 5'-GCTGCTTCCCCTACAAGTTCC-3' and 5'-GCTTTGGCTGCCTGTGTCAGTATGC-3'. The primers for SREBP1a, SREBP1c, fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), peroxisome proliferator-activated receptor (PPAR) α , PPAR γ coactivator 1 α

(PGC1 α), the catalytic subunit of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), Krüppel-like factor 15 (KLF15), and 36B4 were as described (5, 7, 10, 12, 19).

Statistical analysis

Data are presented as means \pm SEM and were compared between or among groups by analysis of variance (ANOVA). A *P* value of <0.05 was considered statistically significant.

RESULTS

Effects of overexpression of STAT3 in the liver on metabolic parameters

Systemic infusion of adenovirus vectors results in liver-specific expression of exogenous genes (4, 10). We therefore forced expression of STAT3 in the liver of 8-week-old *Lepr*^{-/-} mice, which are obese and exhibit insulin resistance and diabetes (6), with the use of an adenovirus vector encoding wild-type STAT3 (AxCASTAT3). Mice were injected with AxCASTAT3 at 8×10^8 or 12×10^8 PFU, with control animals receiving an adenovirus vector encoding β -galactosidase (AxCALacZ) at 12×10^8 PFU. The abundance of STAT3 mRNA in the liver 4 days after injection of mice with AxCASTAT3 at 8×10^8 or 12×10^8 PFU was ~ 2.5 and ~ 13 times that in control mice, respectively (Fig. 1A). The amount of STAT3 mRNA in other organs including adipose tissue and skeletal muscle was not affected by injection of AxCASTAT3 (data not shown). Although body mass was similar among the three groups of mice (Fig. 1B), the blood glucose and plasma insulin concentrations in the randomly fed state were decreased by injection of AxCASTAT3 in a dose-dependent manner (Fig. 1C). These results indicate that hepatic expression not only of an oncogenic form of STAT3 (4) but also of wild-type STAT3 ameliorates hyperglycemia and hyperinsulinemia of insulin-resistant diabetic animals.

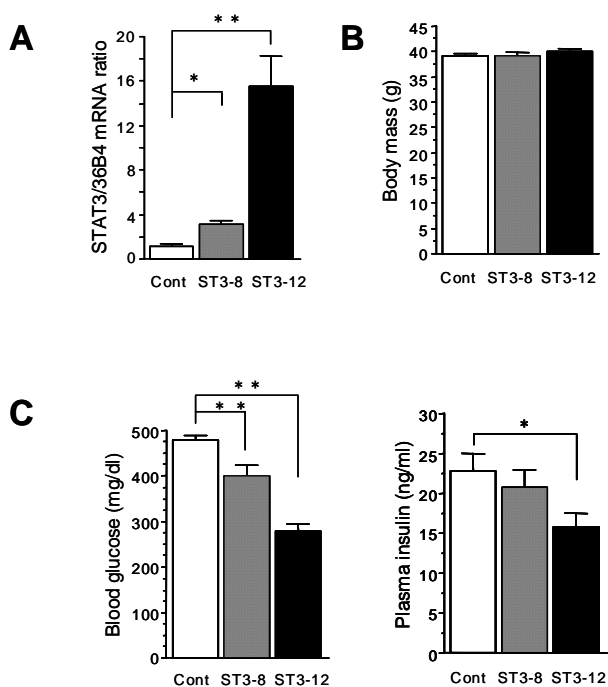


Figure 1.

Effects of hepatic overexpression of STAT3 on body mass and glucose metabolism. The abundance of STAT3 mRNA in the liver (A), body mass (B), as well as blood glucose and plasma insulin concentrations (C) in the randomly fed state were determined 4 days after the injection of 8-week-old *Lepr*^{-/-} mice either with AxCASTAT3 at 8×10^8 PFU (ST3-8) or 12×10^8 PFU (ST3-12) or with AxCALacZ at 12×10^8 PFU (Cont). Data are means \pm SEM of values from six mice. **P* < 0.05 , ***P* < 0.01 for the indicated comparisons.

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We next investigated the effects of AxCASTAT3 on the plasma lipid profile. The plasma level of free fatty acids was similar among the three groups of mice, whereas the plasma triglyceride concentration was significantly increased in mice injected with AxCASTAT3 at 12×10^8 PFU (Fig. 2). The plasma level of total cholesterol was also increased by hepatic expression of STAT3 in a dose-dependent manner, whereas the levels of low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol did not differ significantly among the three groups of animals.

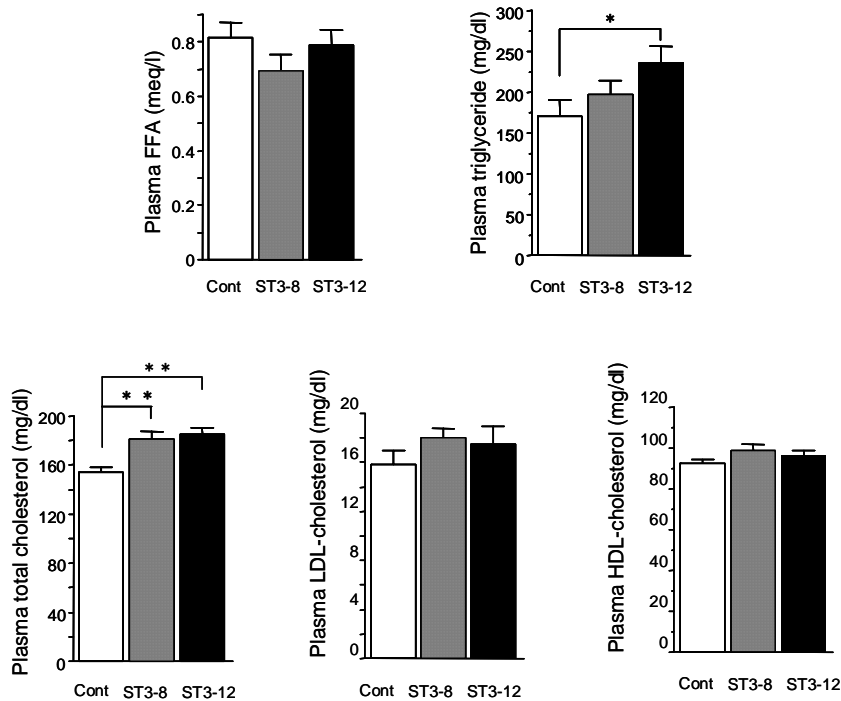


Figure 2. Effects of hepatic overexpression of STAT3 on the plasma lipid profile. Plasma concentrations of free fatty acids (FFA), triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol in the randomly fed state were determined 4 days after the injection of 8-week-old *Lepr*^{-/-} mice either with AxCASTAT3 at 8×10^8 PFU (ST3-8) or 12×10^8 PFU (ST3-12) or with AxCALacZ at 12×10^8 PFU (Cont). Data are means \pm SEM of values from six mice. * $P < 0.05$, ** $P < 0.01$.

Effects of overexpression of STAT3 in the liver on expression of gluconeogenic genes

The hepatic abundance of mRNA for PEPCK, a key enzyme of gluconeogenesis, was significantly reduced in mice injected with AxCASTAT3 (Fig. 3), consistent with our previous finding (4). The abundance of transcripts encoding the catalytic subunit of G6Pase (which catalyzes the final step of gluconeogenesis) or PGC1 α (a transcriptional coactivator of genes for gluconeogenic enzymes) (13, 23) was also decreased in mice injected with AxCASTAT3, although these changes were not statistically significant. There was also a tendency for the amount of mRNA for KLF15, a transcription factor that regulates the expression of gluconeogenic genes (3, 19), to be decreased in mice injected with

AxCASTAT3. The abundance of FOXO1 mRNA was not affected by hepatic overexpression of STAT3.

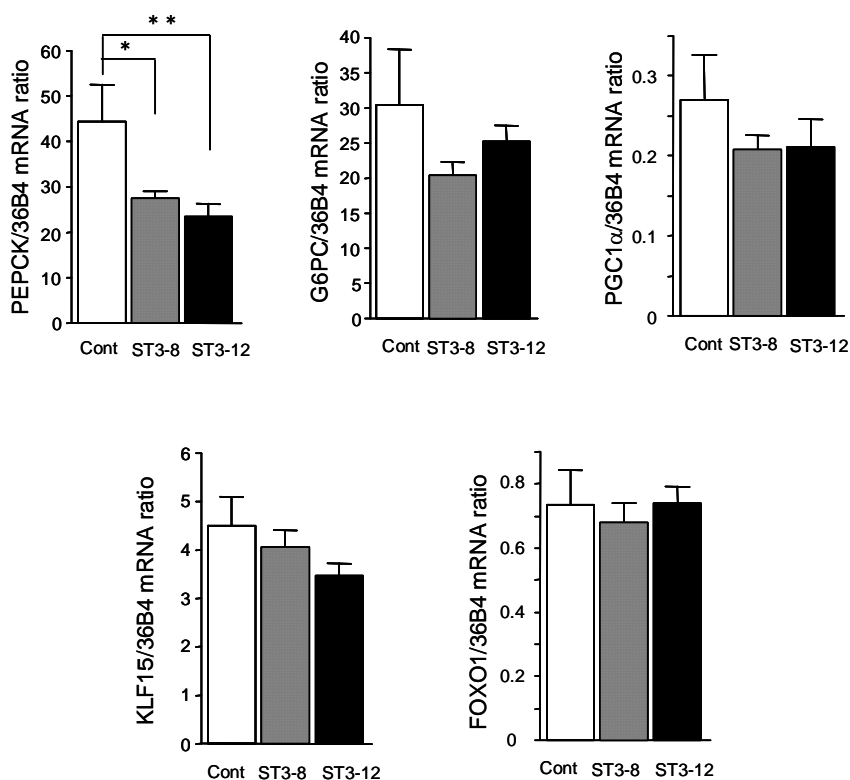


Figure 3. Effects of hepatic overexpression of STAT3 on the expression of gluconeogenic genes in the liver. The hepatic abundance of mRNAs for the indicated proteins (G6PC = catalytic subunit of G6Pase) in the randomly fed state was determined 4 days after the injection of 8-week-old *Lepr^{-/-}* mice either with AxCASTAT3 at 8×10^8 PFU (ST3-8) or 12×10^8 PFU (ST3-12) or with AxCALacZ at 12×10^8 PFU (Cont). Data are means \pm SEM of values from six mice. * $P < 0.05$, ** $P < 0.01$.

Effects of overexpression of STAT3 in the liver of the expression of genes involved in lipid metabolism

The transcription factors SREBP1a and SREBP1c play central roles in the regulation of genes for fatty acid synthesis, whereas SREBP2 is important for the induction of genes that contribute to cholesterol synthesis (2, 16). The hepatic expression of genes for these three transcription factors was not significantly altered by forced expression of STAT3 in the liver, although there was a tendency for the amount of SREBP1c mRNA to be decreased and that of SREBP2 mRNA to be increased by injection of AxCASTAT3 (Fig. 4). In contrast, the amounts of mRNAs for FAS and ACC, both of which catalyze important steps in fatty acid synthesis, were significantly increased by injection of AxCASTAT3 at 12×10^8 PFU. The amounts of mRNAs for SCD1, which also participates in fatty acid synthesis, and for HMGCR, an enzyme that contributes to cholesterol synthesis, were not significantly affected by hepatic overexpression of STAT3. We finally investigated the expression of genes whose products function in β -oxidation. The abundance of ACO mRNA was decreased by injection

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of AxCASTAT3 at 12×10^8 PFU, whereas that of CPT1 or PPAR α mRNAs was not significantly altered by hepatic overexpression of STAT3.

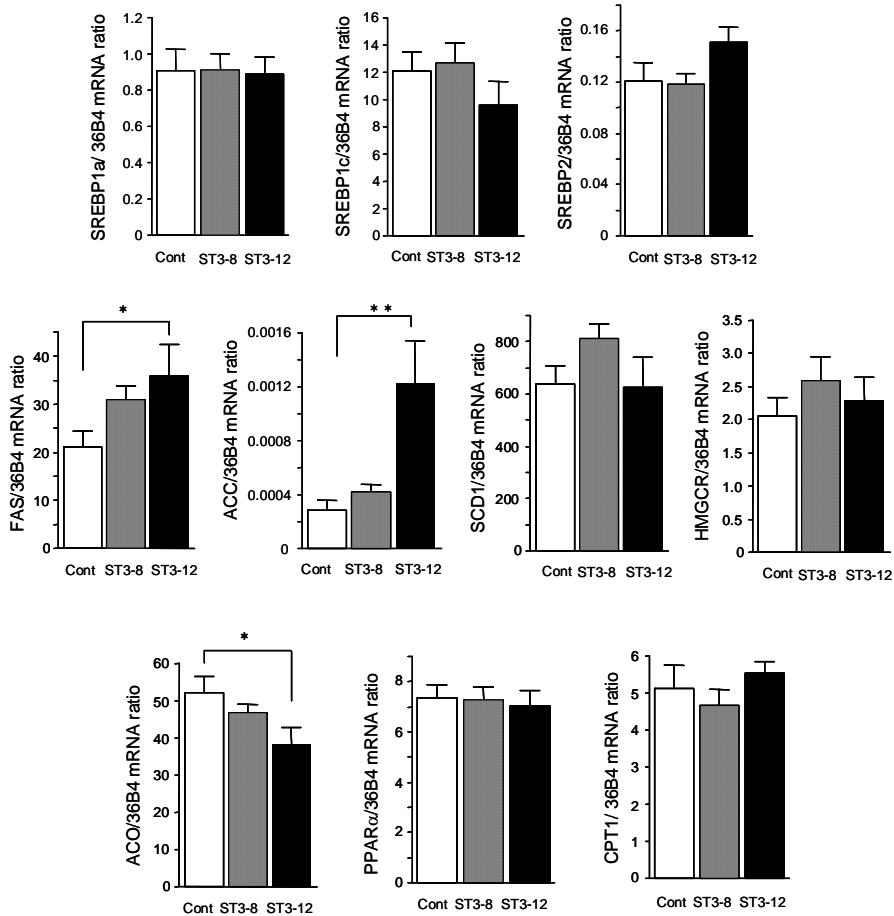


Figure 4. Effects of hepatic overexpression of STAT3 on the hepatic expression of genes involved in lipid metabolism. The hepatic abundance of mRNAs for the indicated proteins in the randomly fed state was determined 4 days after the injection of 8-week-old *Lepr*^{-/-} mice either with AxCASTAT3 at 8×10^8 PFU (ST3-8) or 12×10^8 PFU (ST3-12) or with AxCALacZ at 12×10^8 PFU (Cont). Data are means \pm SEM of values from six mice. * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

The regulation of genes whose products contribute to hepatic nutrient metabolism is a promising therapeutic target for metabolic disorders. Fibrates, for example, reduce the serum triglyceride concentration and serve as ligands for the transcription factor PPAR α (21), whereas the therapeutic action of metformin, one of the most widely prescribed antidiabetes drugs (22), is attributable at least in part to suppression of the expression of gluconeogenic genes (15). We have previously shown that forced expression of a self-dimerized, oncogenic mutant of STAT3 (STAT3-C) specifically in the liver ameliorated the hyperglycemia and

hyperinsulinemia of *Lepr^{-/-}* mice (4). In this previous study, the abundance of STAT3-C was about five times that of endogenous STAT3 (H. Inoue, W. Ogawa, and M. Kasuga, unpublished data). We have now shown that hepatic overexpression of wild-type STAT3 resulting from a ~2.5- or ~13-fold increase in the amount of STAT3 mRNA also reduced blood glucose and plasma insulin concentrations in the same animal model, although the extents of these changes were relatively small compared with those induced by STAT3-C in our previous study.

Forced expression of STAT3-C reduced the hepatic expression of gluconeogenic genes including those for PEPCK, the catalytic subunit of G6Pase, and PGC1 α (4). In the present study, although expression of the PEPCK gene was significantly inhibited by STAT3 overexpression, the reduction in the abundance of mRNAs for the catalytic subunit of G6Pase or PGC1 α was not statistically significant, possibly because the transcriptional regulatory activity of wild-type STAT3 is lower than that of STAT3-C. Given that alteration of the expression of PEPCK alone influences glucose tolerance in mice (18, 20), it is likely that the decrease in the expression of the PEPCK gene contributes to the amelioration of hyperglycemia and hyperinsulinemia in *Lepr^{-/-}* mice by overexpression of wild-type STAT3.

Hepatic overexpression of STAT3 in *Lepr^{-/-}* mice increased the plasma level of total cholesterol without affecting that of HDL- or LDL-cholesterol. These results suggest that the increase in the plasma level of total cholesterol induced by injection of AxCASTAT3 was attributable to an increase in the levels of very low density lipoprotein (VLDL) or intermediate density lipoprotein (IDL), both of which are atherogenic lipoproteins. This notion is also consistent with the observation that the plasma triglyceride concentration was increased by forced expression of STAT3, given that both VLDL and IDL are triglyceride rich. Clinical application of hepatic activation of STAT3 might thus require concomitant administration of lipid-lowering drugs.

Hepatic expression of the genes for the lipogenic enzymes FAS and ACC was increased by forced expression of STAT3 in the liver. Up-regulation of the expression of these enzymes likely contributes to the observed changes in the circulating lipid profile induced by injection of AxCASTAT3. The amounts of mRNAs for SREBP1a and SREBP1c, both of which activate transcription of the genes for FAS and ACC (2, 16), were not significantly altered by overexpression of STAT3, however, suggesting that the regulation of these lipogenic genes by STAT3 is independent of the SREBP1 pathway. Moreover, the abundance of mRNA for ACO, an enzyme involved in the oxidation of fatty acids, was reduced in mice overexpressing STAT3, possibly also contributing to the increase in the levels of triglyceride and cholesterol in these animals.

In summary, we have shown here that overexpression of STAT3 in the liver ameliorated the hyperglycemia and hyperinsulinemia of insulin-resistant diabetic mice. However, it also increased the levels of atherogenic lipoproteins, likely through alteration of the expression of hepatic genes involved in lipid metabolism, including those for FAS, ACC, and ACO. The mechanism by which STAT3 regulates these genes remains to be clarified, but its elucidation may provide a basis for clinical application of activation of the STAT3 signaling pathway to the treatment of metabolic diseases.

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