Overexpression of c-*myc* in diabetic mice restores altered expression of the transcription factor genes that regulate liver metabolism

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Overexpression of the c-Myc transcription factor in liver induces glucose uptake and utilization. Here we examined the effects of c-*myc* overexpression on the expression of hepatocyte-specific transcription factor genes which regulate the expression of genes controlling hepatic metabolism. At 4 months after streptozotocin (STZ) treatment, most diabetic control mice were highly hyperglycaemic and died, whereas in STZ-treated transgenic mice hyperglycaemia was markedly lower, the serum levels of β-hydroxybutyrate, triacylglycerols and non-esterified fatty acids were normal, and they had greater viability in the absence of insulin. Furthermore, long-term STZ-treated transgenic mice showed similar glucose utilization and storage to healthy controls. This was consistent with the expression of glycolytic genes becoming normalized. In addition, restoration of gene expression of the transcription factor, sterol receptor element binding protein 1c, was observed in the livers of these transgenic mice. Further, in STZ-treated transgenic mice the expression of genes involved in the control of gluconeogenesis (phosphoenolpyruvate carbokykinase), ketogenesis (3-hydroxy-3-methylglutaryl-CoA synthase) and energy metabolism (uncoupling protein 2) had returned to normal. These findings were correlated with decreased expression of genes encoding the transcription factors hepatocyte nuclear factor 3γ, peroxisome proliferator-activated receptor α and retinoid X receptor. These results indicate that c-*myc* overexpression may counteract diabetic changes by controlling hepatic glucose metabolism, both directly by altering the expression of metabolic genes and through the expression of key transcription factor genes.

Key words: glucose metabolism, hepatocyte nuclear factor 3γ (HNF3γ), peroxisome proliferator-activated receptor α (PPARα), sterol regulatory element binding protein 1c (SREBP1c), transgenic mice.

INTRODUCTION

The liver has a central role in glucose homoeostasis. When plasma glucose is high, the liver takes it up and replenishes glycogen stores. During starvation, the liver releases glucose into the blood via glycogenolysis and gluconeogenesis [1]. Glucose transport and phosphorylation are the first steps in glucose utilization in the liver [1]. In addition, glucose regulates hepatic gene transcription [2,3]. Although it is clear that glucose phosphorylation by glucokinase (GK) is responsible for the glucose-dependent regulation of gene transcription in hepatocytes, the subsequent steps remain unclear [2–4]. Several transcription factors have been proposed as intermediates in the control of gene transcription by glucose. Sterol regulatory element binding protein 1c (SREBP1c) has been suggested as a candidate transcription factor for the actions of both glucose and insulin on hepatic genes involved in glycolysis and lipogenesis [5,6]. In addition, peroxisome proliferator-activated receptor α (PPARα) [7,8], retinoid X receptor (RXR) [9,10] and members of the hepatocyte nuclear factor (HNF) family [11–13] have a central function in regulating the hormonal and metabolic control of expression of key genes in many physiological processes, such as glucose, fatty acid and cholesterol metabolism in the liver.

c-Myc belongs to the basic helix–loop–helix family of transcription factors which bind to the enhancer box 5′-CACGTG-3′ [14–16]. This sequence is contained in the glucose/carbohydrate regulatory elements located in the promoters of several genes coding for enzymes involved in hepatic glycolysis and lipogenesis [17,18]. Previous studies showed that overexpression of c-*myc* leads to an induction of hepatic glycolysis by increasing the expression and activity of genes coding for key regulatory enzymes of glycolysis, such as GK, L-type pyruvate kinase (L-PK) and 6-phosphofructo-2-kinase (PFK-2) [19,20]. Thus c-Myc may have a physiological role in the control of liver carbohydrate metabolism *in vivo* [19–21].

Type I diabetes results from the autoimmune destruction of the insulin-producing β-cells of the pancreas, and is characterized by a lack of insulin, which leads to the development of severe hyperglycaemia [22,23]. Diabetic hyperglycaemia can be reduced by increased glucose transport and phosphorylation by key target tissues, such as liver and skeletal muscle. We showed previously that overexpression of c-Myc in the livers of transgenic mice leads to counteraction of short-term (7 days) diabetic alterations following streptozotocin (STZ) treatment [24]. This is achieved through c-Myc inducing the expression of key genes involved in hepatic glucose uptake and utilization, and blocking the activation of gluconeogenesis and ketogenesis, in the absence of cell proliferation and transformation [24]. The effects of c-Myc are noted in both healthy and short-term STZ-treated transgenic mice [19,24], suggesting that this transcription factor re-inforces the effects of insulin in healthy mice, while it mimics the effects of the hormone in diabetes, when insulinaemia is low. Thus, in

Abbreviations used: C/EBP, CCAAT/enhancer binding protein; GK, glucokinase; GLUT2, glucose transporter 2; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HNF, hepatocyte nuclear factor; L-PK, L-type pyruvate kinase; NEFA, non-esterified fatty acids; PEPCK, phosphoenolpyruvate carboxykinase; PFK-2, 6-phosphofructo-2-kinase; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SREBP, sterol regulatory element binding protein; STZ, streptozotocin; UCP, uncoupling protein.

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the livers of these transgenic mice, c-Myc might be considered a common step in the transcriptional regulation of hepatic glucose metabolism genes by glucose and insulin.

To determine the mechanism(s) by which c-Myc counteracts diabetic alterations, here we have examined the effects of overexpressing c-*myc* on the expression of hepatocyte-specific transcription factor genes that in turn regulate the expression of genes involved in the control of liver glucose metabolism. We found a direct relationship between c-*myc* overexpression and restoration of the expression of transcription factor genes that control hepatic glucose metabolism.

EXPERIMENTAL

Treatment of mice

The development and characterization of transgenic mice expressing the phosphoenolpyruvate carboxykinase (PEPCK)/c-*myc* chimaeric gene were described previously [19]. Heterozygous transgenic male mice (C57Bl6/SJL) aged 3–4 months [19] were fed *ad libitum* on a standard diet (Panlab, Barcelona, Spain) and maintained under a light/dark cycle of 12 h/12 h (lights on at 08:00 h). When stated, mice were starved for 16 h. Animals were killed and liver samples were taken between 09:00 and 10:00 h. Diabetes was induced by injection through the jugular vein of a dose of 2 mg of STZ/10 g body weight on two consecutive days. STZ (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in a 10 mM sodium citrate solution containing 0.9 % NaCl, pH 4.5, immediately before administration. Diabetes was assessed by measuring glycaemic and glucosuric levels (Glucometer® and Gluketur Test; Bayer, Leverkusen, Germany). All experimental procedures involving mice were approved by the Ethics and Experimental Animal Committee of the Universitat Autònoma de Barcelona.

Northern blot analysis

Total RNA was obtained from liver by the guanidine isothiocyanate method [25]. Aliquots of 30 μg of total liver RNA were electrophoresed on a 1 % (w/v) agarose gel containing 2.2 M formaldehyde and then transferred to nylon membranes by capillary blotting. Northern blots were hybridized, as previously described [19,24], to the following cDNA probes: c-Myc, GK, PFK-2, L-PK, GLUT2 (glucose transporter 2), PEPCK, mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and β-actin [19,24]; SREBP1c (provided by B. Spiegelman, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, U.S.A.); the RXR heterodimeric partner genes mouse retinoic acid receptor (RAR) and RXR [provided by P. Chambon, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch (Strasbourg), France]; mouse PPARα (provided by P. Tontonoz, Howard Hughes Medical Institute, UCLA School of Medicine, Los Angeles, CA, U.S.A.); human uncoupling protein 2 (UCP2; provided by J. P. Giacobino, Centre Médical Universitaire, Geneva, Switzerland); HNF3α, β and γ (provided by K. Kaestner, University of Pennsylvania, School of Medicine, Philadelphia, PA, U.S.A.); and CCAAT/enhancer binding protein (C/EBP) α and β (provided by S. McKnight, UT Southwestern Medical Center, Dallas, TX, U.S.A.). These probes were labelled using [α-^{32}P]dCTP, following the method of random oligopriming as described by the manufacturer (Roche Molecular Biochemicals, Mannheim, Germany). The specific radioactivity of the DNA probe thus labelled was approx. 10^9 c.p.m./μg of DNA. Membranes were placed in contact with Kodak XAR-5 films (Rochester, NY, U.S.A.).

The amount of mRNA expressed in individual samples was quantified by densitometry and then standardized to the amount of β-actin. Means $\pm$ S.D. for at least five samples were calculated to validate the statistical significance of observed changes (using the Student–Newmann–Keuls test). Differences were considered statistically significant at $P < 0.05$.

Hormone, enzyme and metabolite assays

To determine enzyme activities and the concentrations of metabolites, liver samples were clamped, frozen *in situ* and kept at -80 °C until analysis. Hepatic GK and L-PK enzyme activities were analysed in liver samples as described previously [19,24]. The concentrations of glycogen, glucose 6-phosphate and lactate were measured in perchloric acid extracts as described elsewhere [19,24]. The glucose concentration in blood was determined by using a Glucometer Elite® instrument (Bayer) according to the manufacturer's instructions. Serum non-esterified fatty acids (NEFA) were measured by the acyl-CoA synthase/acyl-CoA oxidase method (Wako Chemicals). β-Hydroxybutyrate levels in serum were measured by the β-hydroxybutyrate dehydrogenase technique (Roche Molecular Biochemicals). Serum triacylglycerols were determined enzymically (GPO-PAP; Roche Molecular Biochemicals). Insulin levels in serum were determined by RIA (CIS Biointernational), using rat insulin (Eli Lilly, Indianapolis, IN, U.S.A.) as standard.

Serum parameters, enzyme activities and metabolite concentrations are expressed as means $\pm$ S.E.M. Statistical differences between data were analysed using the Student–Newmann–Keuls test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Decrease in diabetic hyperglycaemia in transgenic mice overexpressing c-Myc in the long term after the destruction of pancreatic β-cells with STZ

To determine whether the overexpression of c-Myc in the liver led to the long-term counteraction of diabetic alterations, control and transgenic mice were treated with STZ. At 1 month after STZ treatment, fed control mice had high levels of blood glucose (> 600 mg/dl; the upper limit of detection) and remained highly hyperglycaemic afterwards (Table 1). In contrast, fed STZ-treated transgenic mice showed an approx. 2.5-fold increase in glycaemia 1 month after STZ treatment, but only a 3-fold increase in blood glucose levels 4 months after STZ treatment

Table 1 Blood glucose levels in STZ-treated mice

Blood glucose levels were measured in fed and in overnight-starved control and transgenic mice before and after STZ treatment, as described in the Experimental section. Results are means $\pm$ S.E.M. For the STZ control mice, $n = 60$ at day 0 and $n = 6$ at 4 months; for the STZ transgenic mice, $n = 38$ at day 0 and $n = 20$ at 4 months (*$P < 0.05$, **$P < 0.01$ compared with STZ-treated controls).

		Glucose (mg/dl)		
Mice	Time (months) ...	0	1	4
Fed				
Control		159 $\pm$ 13	> 600	> 600
Transgenic		145 $\pm$ 11	324 $\pm$ 43*	398 $\pm$ 61*
Fasted				
Control		91 $\pm$ 9	194 $\pm$ 23	229 $\pm$ 32
Transgenic		82 $\pm$ 8	103 $\pm$ 18*	116 $\pm$ 20**

(Table 1). At this time, very low levels of circulating insulin were found in all STZ-treated mice (< 0.2 ng/ml). This suggested that, in the absence of insulin treatment, overexpression of c-Myc partially counteracted and delayed severe hyperglycaemia (Table 1). Furthermore, while starvation increased glycaemia by approx. 2.5-fold in control diabetic mice, starved STZ-treated transgenic mice showed similar blood glucose levels to starved non-STZ-treated controls (Table 1).

The partial counteraction of diabetic hyperglycaemia observed in the long term after STZ treatment of transgenic mice was paralleled by improved survival and increased body weight. STZ-treated control mice showed a marked loss (approx. 25 %) of body weight (from 29.1 ± 0.5 g to 22.2 ± 1.2 g) and progressively died (only around 10 % remained alive 4 months after treatment with STZ; $n = 60$ at day 0 to $n = 6$ at day 120). In contrast, STZ-treated transgenic mice were viable for longer, and approx. 52 % remained alive 4 months after STZ treatment (from $n = 38$ at day 0 to $n = 20$ at day 120); they also maintained their body weight (from 28.0 ± 0.6 g to 28.2 ± 1.3 g; $P < 0.05$). Furthermore, the livers of long-term STZ-treated transgenic mice did not show morphological alterations by histological analysis (results not shown). STZ-treated transgenic mice also showed normal concentrations of serum β-hydroxybutyrate (control, 0.72 ± 0.1 mmol/l; STZ-control, 3.36 ± 0.5 mmol/l; STZ-transgenic, 0.96 ± 0.2 mmol/l), triacylglycerols (control, 153 ± 13 mg/dl; STZ-control, 374 ± 21 mg/dl; STZ-transgenic, 176 ± 15 mg/dl), and NEFA (control, 0.76 ± 0.1 mmol/l; STZ-control, 1.93 ± 0.3 mmol/l; STZ-transgenic, 0.89 ± 0.15 mmol/l). Thus, without insulin, transgenic mice showed restoration of serum parameters, probably as a consequence of the effects of c-Myc on hepatic glucose and lipid metabolism.

Restoration of the expression/activity of key genes/proteins involved in the control of hepatic glucose metabolism

To determine the expression of key genes involved in the regulation of hepatic glucose metabolism, total RNA was obtained from the livers of control and transgenic mice 4 months after STZ treatment. Two mRNA transcripts were detected in the livers of STZ-treated transgenic mice when Northern blots were hybridized with a c-Myc probe (Figure 1) [19,24]. Very low levels of expression of the genes encoding GK, PFK-2 and L-PK were detected in the livers of STZ-treated control mice, whereas STZ-treated transgenic mice expressed high levels of GK (approx. 11-fold greater; $P < 0.01$), PFK-2 (approx. 5-fold; $P < 0.05$) and L-PK (approx. 4-fold; $P < 0.05$) mRNAs (Figure 1). Furthermore, the GK, PFK-2 and L-PK mRNA levels observed in the livers of STZ-treated transgenic mice were similar to those observed in healthy control mice (Figure 1).

The increases noted in these mRNA levels were paralleled by increases in enzyme activities. Thus, 4 months after STZ treatment, transgenic mice showed normal GK and L-PK activities [GK: STZ-control, 3 ± 1 m-units/mg of protein; STZ-transgenic, 20 ± 2 m-units/mg ($P < 0.01$); L-PK: STZ-control, 0.06 ± 0.03 units/mg; STZ-transgenic, 0.21 ± 0.03 units/mg ($P < 0.05$)]. The normalization of GK and L-PK enzyme activities in the livers of STZ-treated transgenic mice was associated with normal concentrations of glucose 6-phosphate (STZ-control, 36 ± 6 nmol/g of liver; STZ-transgenic, 207 ± 13 nmol/g; $P < 0.01$), glycogen (STZ-control, 4 ± 1 mg/g of liver; STZ-transgenic, 41 ± 5 mg/g; $P < 0.01$) and lactate (STZ-control, 0.02 ± 0.01 μmol/g of liver; STZ-transgenic, 0.15 ± 0.04 μmol/g; $P < 0.01$). Thus the decrease in hyperglycaemia observed in STZ-treated transgenic mice probably resulted, in part, from increased hepatic glucose utilization and storage.

Restored expression of the transcription factor SREBP1c in the livers of STZ-treated transgenic mice

The SREBP1c transcription factor regulates glycolytic and lipogenic genes [5,6]. At 4 months after STZ treatment, low levels of SREBP1c mRNA were detected in the livers of control mice. In contrast, the increase in c-*myc* gene expression in the livers of STZ-treated transgenic mice was paralleled by an 11-fold increase in the levels of SREBP1c ($P < 0.01$) (Figure 1). This concentration of SREBP1c mRNA was similar to that in the livers of healthy control mice (Figure 1). Thus overexpression of c-Myc in the livers of STZ-treated transgenic mice up-regulated and restored SREBP1c gene expression.

Decreased gene expression of the regulatory enzymes of gluconeogenesis and ketone body synthesis in the livers of STZ-treated transgenic mice

The expression of the PEPCK and mitochondrial HMG-CoA synthase genes is markedly induced by diabetes [26,27]. STZ-treated transgenic mice showed markedly lower (approx. 6-fold; $P < 0.01$) expression of these two enzymes than diabetic controls (Figure 1). Moreover, the decrease in the expression of PEPCK in the livers of STZ-treated transgenic mice was paralleled by a decrease (approx. 6-fold; $P < 0.01$) in GLUT2 mRNA levels, which increased in the livers of STZ-treated control mice (Figure 1). The expression of these genes in the livers of STZ-treated transgenic mice was similar to that observed in healthy control mice. These findings were related to the decrease in hyperglycaemia and ketone body levels detected in the serum of STZ-treated transgenic mice.

Down-regulation of RXR and PPARα gene expression in the livers of STZ-treated transgenic mice

We further examined the effects of overexpression of c-Myc in the livers of STZ-treated transgenic mice by studying the expression of nuclear receptor genes that control liver metabolism. The RAR family belongs to the nuclear hormone receptor superfamily and consists of two classes of retinoid receptors, RAR and RXR [9,10]. These two nuclear receptor families each comprise three subtypes, referred to as α, β and γ [9,10]. At 4 months after STZ treatment, the expression of RXRα was approx. 7-fold lower ($P < 0.01$) in the livers of transgenic mice than in the livers of diabetic control mice (Figure 2). Moreover, the expression of RXRβ and RXRγ was also less (approx. 2.5-fold and 3-fold respectively; $P < 0.05$) in the livers of STZ-treated transgenic mice (Figure 2). The level of expression of these genes was similar to that observed in healthy control mice (Figure 2). In addition, the level of mRNA of RARα decreased (by approx. 3-fold; $P < 0.05$) to control levels in the livers of STZ-treated transgenic mice (Figure 2). However, the expression of RARβ and RARγ was unchanged in the livers of STZ-treated mice (Figure 2).

PPARs also belong to the nuclear hormone receptor superfamily [7,8]. The PPARα isoform is involved both in activating gluconeogenesis and in the catabolism of fatty acids in the liver through PPAR/RXR heterodimers [7,8]. PPARα expression was lower (approx. 5-fold; $P < 0.01$) in the livers of STZ-treated transgenic mice than in STZ-treated control mice, returning to normal levels (Figure 2). All these results suggested that, in the long term after STZ treatment, overexpression of c-Myc in the livers of transgenic mice decreased the expression of the PPARα and RXR genes. Restoration of the expression of these two genes in the livers of STZ-treated transgenic mice

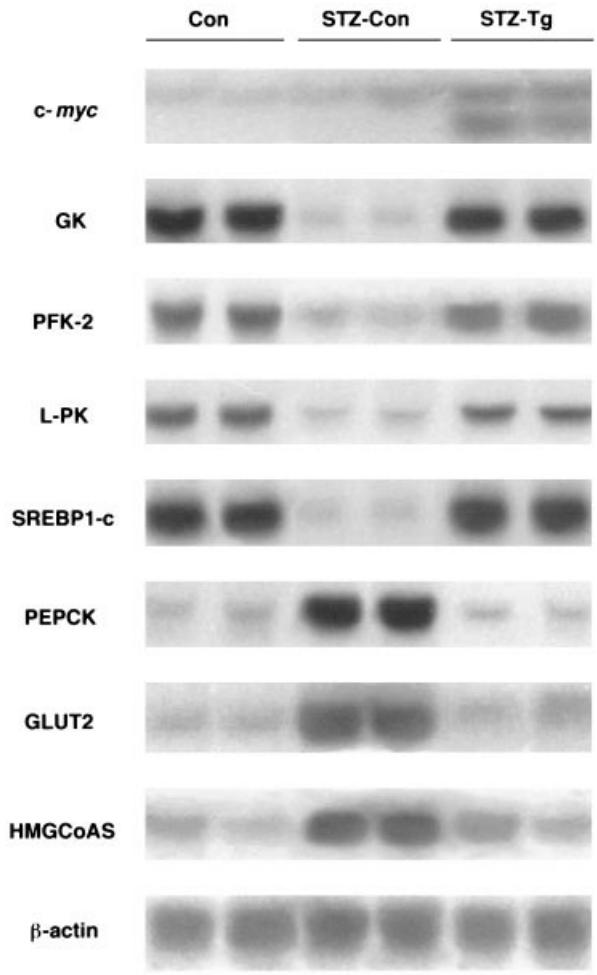

Figure 1 Expression of c-*myc* and key genes that regulate glycolysis, gluconeogenesis, glucose transport and ketone body metabolism

Total RNA was obtained from the livers of untreated control mice (Con) and STZ-treated control (STZ-Con) and transgenic (STZ-Tg) mice, and analysed by Northern blot analysis as indicated in the Experimental section. A representative Northern blot hybridized with probes for c-*myc*, GK, PFK-2, L-PK, SREBP1c, GLUT2, PEPCK, mitochondrial HMG-CoA synthase (HMGCoAS) and β-actin is shown.

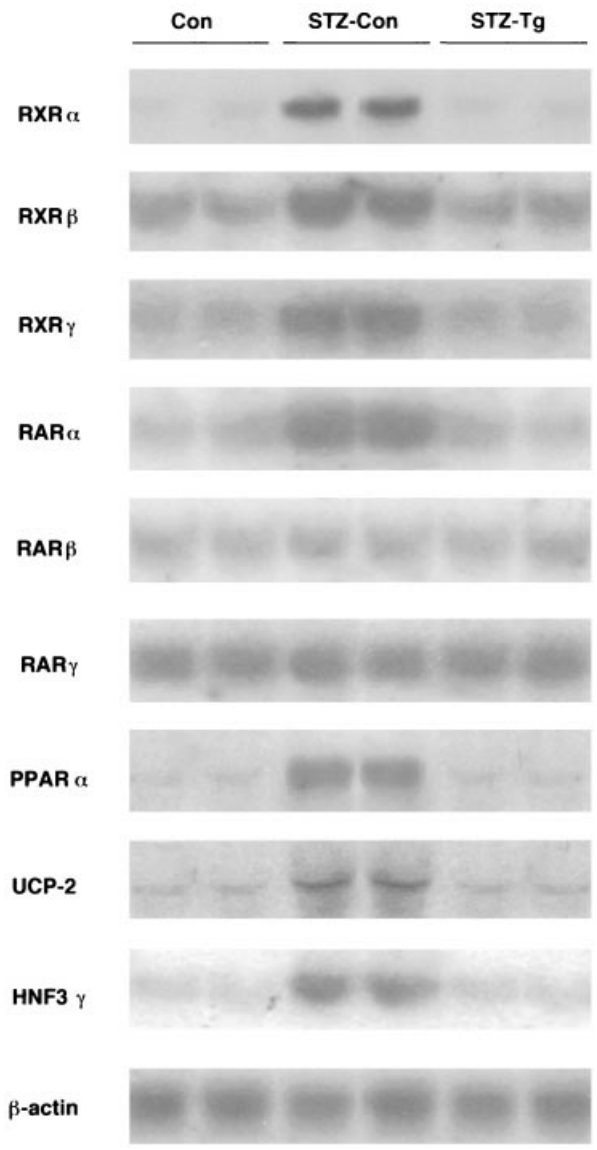

Figure 2 Expression of nuclear hormone receptor superfamily genes

Total RNA was obtained from the livers of untreated control mice (Con) and STZ-treated control (STZ-Con) and transgenic (STZ-Tg) mice, and analysed as indicated in the Experimental section. A representative Northern blot hybridized with probes for retinoid receptor genes, PPARα, UCP2, HNF3γ and β-actin is shown.

probably contributed to the normalization of serum triacylglycerol and cholesterol levels observed in these mice.

PPARs are also involved in the control of energy homoeostasis [7,8]. PPARα regulates UCP2 gene expression in the liver [28]. The expression of UCP2 was approx. 6-fold lower ($P < 0.01$) in the livers of STZ-treated transgenic mice than in diabetic control mice (Figure 2). This paralleled the normalization of the expression of the PPARα and RXR genes. Thus the decrease in the expression of these two genes may contribute to the normalization of carbohydrate, lipid and energy metabolism observed in STZ-treated transgenic mice.

Effects of c-Myc overexpression on HNF genes in the livers of STZ-treated transgenic mice

The HNFs activate and/or repress the transcription of a large number of genes that are critical for diverse biological processes, such as development, differentiation and metabolism [11–13]. The expression of different families of HNF genes, including HNF3, HNF4 and C/EBP, was also analysed in the livers of STZ-treated control and transgenic mice. STZ-treated transgenic mice showed a decrease (approx. 7-fold; $P < 0.01$) in the expression of HNF3γ (Figure 2). The expression of this gene in the livers of the STZ-treated transgenic mice was similar to that observed in healthy control mice (Figure 2). In contrast, differences observed in the expression of HNF3α, HNF3β, C/EBPα, C/EBPβ and HNF4 between STZ-treated control and transgenic mice genes were statistically insignificant (results not shown).

DISCUSSION

Previous studies have shown that c-Myc has a physiological function in the control of carbohydrate metabolism [19–21]. The

present study describes the ability of c-*myc* overexpression to counteract diabetic alterations in the long term after the destruction of pancreatic β-cells by STZ, and its effects on the expression of specific transcription factor genes involved in the control of liver metabolism. At 4 months after STZ treatment, the overexpression of c-Myc in the livers of transgenic mice was able to partially counteract diabetic hyperglycaemia when mice were fed, and to lead to normoglycaemia during starvation. This was most probably achieved through the ability of c-Myc both to induce hepatic glucose uptake and utilization and to block the activation of gluconeogenesis. The improvements in the hepatic metabolism of STZ-treated transgenic mice also resulted in longer survival and the maintenance of body weight. Previous studies have shown that STZ-treated transgenic mice overexpressing GK in the liver had increased hepatic glucose metabolism, decreased gluconeogenesis and lower diabetic hyperglycaemia [29,30]. Similarly, restoration of the expression and activity of key genes involved in the control of hepatic glucose metabolism, such as those encoding GK, L-PK and PFK-2, was observed in the livers of short-term [24] and long-term STZ-treated transgenic mice overexpressing c-Myc. In the promoter region of the GK, L-PK and PFK-2 genes, E-boxes are bound and activated by c-Myc [31–33]. Similarly, insulin administration in mice with STZ-induced diabetes led to a restoration of the expression of these genes [34,35].

SREBP1c has been described as a mediator of insulin action in the liver, since it induces glycolytic and lipogenic genes, such as GK [5,6]. The expression of the SREBP1c gene is regulated by glucose and insulin [36]. An E-box motif, 5′-CACGTG-3′, has also been located in the SREBP1c promoter region [37]. Increased expression of SREBP1c was noted in the livers of STZ-treated transgenic mice. Similarly, SREBP1c mRNA levels fall in rats treated with STZ, and insulin administration reverses this effect [38]. Thus overexpression of c-Myc in the livers of STZ-treated transgenic mice was probably able, similar to insulin, to up-regulate SREBP1c gene expression, which in turn could lead to the expression and activity of the enzymes involved in the control of hepatic glucose utilization.

During the development of diabetes, the activity of the gluconeogenic pathway in the liver increases markedly [26]. PEPCK catalyses a limiting step in gluconeogenesis, and is regulated at the transcriptional level [26]: fasting and diabetes increase PEPCK gene expression, while insulin inhibits it [26]. Similarly, decreased expression of PEPCK was noted in the livers of STZ-treated transgenic mice overexpressing c-Myc. An E-box motif, CACCTG, which can be bound by members of the c-Myc family, has been described in the PEPCK promoter [39]. Furthermore, members of the HNF transcription factor family that regulate PEPCK gene transcription were described [40,41]. Normal expression of PEPCK requires mainly the HNF3γ isoform [40,42]. HNF3γ activates gluconeogenic enzymes to prevent hypoglycaemia during fasting [43]. Its expression increases during diabetes and is down-regulated by insulin [43]. The decrease in the expression of HNF3γ detected in the livers of STZ-treated transgenic mice overexpressing c-*myc* might be related to the observed down-regulation of the expression of PEPCK. However, neither a regulatory element responsive to insulin nor an E-box motif that binds the c-*myc* family of transcription factors has yet been described in the HNF3γ promoter. Thus, in the absence of insulin, the increase in c-Myc appeared to mimic the effects of the hormone on the expression of PEPCK, acting either directly or via inhibition of HNF3γ gene expression. Expression of other HNF genes (HNF3α, HNF3β, HNF4, C/EBPα and C/EBPβ) was unchanged, suggesting that these transcription factors are probably not involved in the long-term control of PEPCK gene expression after STZ treatment.

RXRα has been described as the main heterodimeric partner of PPARα in hepatocytes [44]. PPARα is involved in the activation of hepatic gluconeogenesis, whereas RXRα has recently been described as the main heterodimeric integrator of cholesterol and fatty acid metabolism in the liver [7,8,44]. PPARα gene expression is up-regulated by fatty acids, glucocorticoids and diabetes, while it is down-regulated by insulin [45]. RXRα gene expression is also regulated positively by fatty acids and negatively by insulin [46]. Similarly, the expression of the PPARα and RXRα genes was decreased in the livers of STZ-treated transgenic mice, suggesting that c-Myc might regulate their expression in an insulin-like manner. Recently, inducible hypoxia factor 1 has been described to repress PPARα gene expression through an E-box motif located in the promoter of this gene [47]. Thus c-Myc might also repress the expression of PPARα by binding to this E-box. However, neither a regulatory element responsive to insulin nor an E-box motif has yet been described in the promoter of the RXRα gene.

PPARs bind to specific PPAR response elements in the promoter regions of target genes, through co-operative interactions with RXRs, to regulate the transcription of genes involved in whole-body metabolism [7,8]. PPAR response elements have also been identified in the promoters of the HMG-CoA synthase and GLUT2 genes [48,49]. A decrease in the expression of these two genes was noted in the livers of STZ-treated transgenic mice, which may be related to the decrease in the expression of the PPARα and RXRα genes. Moreover, the decrease in the expression of GLUT2 in the livers of STZ-treated transgenic mice was paralleled by a decrease in PEPCK mRNA. Thus the decreased expression of the GLUT2, PEPCK and HMG-CoA synthase genes may have contributed to a decrease in hepatic glucose and ketone body production, and to the reduction of hyperglycaemia and serum ketone body levels, in the STZ-treated transgenic mice. Furthermore, glucose and ketone body production from gluconeogenic precursors in primary culture was lower in hepatocytes from STZ-treated transgenic mice overexpressing c-*myc* than in those from STZ-treated controls, and similar to that in hepatocytes from healthy control mice [24]. This indicates that dysregulation of gluconeogenic flux and ketone body production is reversed by c-*myc* overexpression.

A decrease in HMG-CoA synthase and GLUT2 gene expression has also been observed in diabetic rats after administration of insulin or treatment with the insulin-mimic vanadate [50,51]. These rats showed normal ketone body levels in serum [50]. Although no E-boxes have been described in the HMG-CoA synthase and GLUT2 genes, we cannot rule out a possible direct effect of c-Myc in restoring the expression of these genes. However, while c-Myc transactivation occurs through binding to E-boxes, transrepression of c-Myc appears to occur by mechanisms that do not require the presence of an E-box [52,53].

Animals with STZ-induced diabetes are vulnerable to cold stress due to an impairment of thermogenesis [54]. UCP2 is involved in the control of thermogenesis and is expressed in numerous tissues, such as white and brown adipose tissue, skeletal muscle and liver [55–57]. In a previous study, hepatic expression of UCP2 was unchanged in diabetic rats 2 days after STZ treatment [58]. However, 4 months after STZ treatment, hepatic UCP2 gene expression was higher in diabetic control mice than in STZ-treated transgenic mice, probably as a defence against hypothermogenesis caused by long-term diabetes. It has been shown that PPARα activation in mice is sufficient to induce hepatic UCP2 gene expression [28]. The decrease in the expression of UCP2 noted in the livers of STZ-treated transgenic mice

overexpressing c-*myc* paralleled the decrease in the expression of PPARα and RXRα genes.

In summary, our results indicate that overexpression of c-Myc was able to counteract diabetic alterations by restoring the expression and activity of key enzymes involved in the control of hepatic carbohydrate, lipid and energy metabolism. This was also related to normalization of the expression of transcription factor genes involved in the control of these metabolic pathways. Thus the present study suggests that c-*myc* may exert effects directly at the level of expression of enzymes involved in the control of metabolic pathways, as well as at the level of the transcription factors that regulate their expression.

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