Rapid Publication

Adenovirus-Mediated Overexpression of Sterol Regulatory Element Binding Protein-1c Mimics Insulin Effects on Hepatic Gene Expression and Glucose Homeostasis in Diabetic Mice

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In vitro, the transcription factor sterol regulatory element binding protein-1c (SREBP-1c) mimics the positive effects of insulin on hepatic genes involved in glucose utilization, such as glucokinase (GK) and enzymes of the lipogenic pathway, suggesting that it is a key factor in the control of hepatic glucose metabolism. Decreased glucose utilization and increased glucose production by the liver play an important role in the development of the hyperglycemia in diabetic states. We thus reasoned that if SREBP-1c is indeed a mediator of hepatic insulin action, a hepatic targeted overexpression of SREBP-1c should greatly improve glucose homeostasis in diabetic mice. This was achieved by injecting streptozotocin-induced diabetic mice with a recombinant adenovirus containing the cDNA of the mature, transcriptionally active form of SREBP-1c. We show here that overexpressing SREBP-1c specifically in the liver of diabetic mice induces GK and lipogenic enzyme gene expression and represses the expression of phosphoenolpyruvate carboxykinase, a key enzyme of the gluconeogenic pathway. This in turn increases glycogen and triglyceride hepatic content and leads to a marked decrease in hyperglycemia in diabetic mice. We conclude that SREBP-1c has a major role in vivo in the long-term control of glucose homeostasis by insulin. ***Diabetes*** **50:2425–2430, 2001**

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FAS, fatty acid synthase; GK, glucokinase; PEPCK, phosphoenolpyruvate carboxykinase; S14, Spot 14; SREBP-1c, sterol regulatory element binding protein-1c; STZ, streptozotocin.

Sterol regulatory element binding protein-1c (SREBP-1c) belongs to a family of transcription factors involved in cholesterol and fatty acid metabolism (1). It is synthesized as a precursor form anchored in endoplasmic reticulum and nuclear membranes. After proteolytic cleavage, its mature active form migrates into the nucleus, where it can bind both sterol regulatory elements and E-boxes (1,2). Some evidence suggests that this transcription factor mediates the effects of insulin on hepatic genes involved in glucose and lipid metabolism. In the liver, its expression is itself stimulated by insulin, and this results in an increased abundance of the precursor and nuclear forms (3–6). In cell lines, overexpression of this factor mimics the effect of insulin on the promoter of the LDL receptor (7) and the fatty acid synthase (FAS) genes (8). More direct evidence was obtained recently using adenoviral vectors expressing either a dominant-negative or a dominant-positive form of SREBP-1c. In primary cultured hepatocytes, the dominant-negative SREBP-1c blocks the ability of insulin to induce insulin-responsive genes, such as glucokinase (GK), a key enzyme of hepatic glucose utilization, or lipogenesis-related proteins (e.g., FAS, acetyl-CoA carboxylase, and Spot 14 [S14]), whereas a dominant-positive form stimulates their expression in the absence of insulin, which is otherwise necessary for their active transcription (4,9). SREBP-1c could then play a pivotal role in hepatic carbohydrate metabolism by mediating the inductive effects of insulin on genes involved in glucose utilization (10).

Alterations in hepatic glucose metabolism play an important role in the development of the hyperglycemia observed in diabetic states. During diabetes, the expression and activity of GK are low as a result of either a lack of insulin (type 1 diabetes) or hepatic insulin resistance (type 2 diabetes), and the liver is unable to metabolize glucose (11,12). Conversely, the expression of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme involved in hepatic gluconeogenesis, is greatly increased in diabetic states, leading to increased glucose production (13). Overexpression of PEPCK in transgenic mice leads to alterations in glucose homeostasis that bear similarities to

type 2 diabetes (14). In contrast, overexpression of hepatic GK in diabetic mice improves glucose homeostasis (15).

We then reasoned that if SREBP-1c is really mimicking the positive effects of insulin on gene expression, a hepatic targeted overexpression of this factor should greatly improve glucose homeostasis in diabetic mice. This was achieved by injecting streptozotocin (STZ)-induced diabetic mice with a recombinant adenovirus containing the cDNA of the mature, transcriptionally active form of SREBP-1c. We show here that overexpression of SREBP-1c in the liver of diabetic mice induces GK and lipogenic enzyme gene expression, represses PEPCK expression, and leads to a dramatic decrease in hyperglycemia, thus mimicking insulin action.

RESEARCH DESIGN AND METHODS

All procedures were carried out according to the French guidelines for the care and use of experimental animals. Male 10-week-old CBA mice (Janvier Breeding Laboratories, Le Genest-St-Isle, France) were housed at 20°C with light from 0700 to 1900, with free access to water and food (UAR, Villemoisson sur Orge, France). Diabetes was induced by a single intraperitoneal injection of STZ (250 mg/kg body wt; Sigma, St. Louis, MO). STZ was dissolved in 50 mmol/l sodium citrate buffer (pH 4) immediately before administration. Diabetes was assessed by both blood sampling from the tail vein and plasma glucose as well as by the presence of overt glycosuria.

Recombinant adenoviruses. The adenovirus vector containing the transcriptionally active NH_2-terminal fragment (amino acids 1–403) of SREBP-1c was constructed according to He et al. (16). Briefly, the cDNA of the transcriptionally active fragment of SREBP-1c was subcloned into the shuttle vector pAd Track-CMV. The resultant plasmid was linearized by the restriction endonuclease *Pme*I and cotransformed with the supercoiled adenoviral vector pAd-Easy1 into *Eschericia coli* strain BJ5183. Recombinants were selected by kanamycin resistance and screened by restriction endonuclease digestion. Then, the recombinant adenoviral construct was cleaved with *Pac*I and transfected into the packaging cell line 293. The adenovirus vector containing the major late promoter with no exogenous gene (null adenovirus) was used as a control. The adenoviral vectors were propagated in 293 cells, purified by cesium chloride density centrifugation, and stored as previously described (9).

Injection of recombinant adenovirus and insulin administration. STZ-induced diabetic mice were treated with either the recombinant adenovirus or insulin. Recombinant adenoviruses were injected to mice anesthetized with halothane (Belamont Veterinary, Paris) via the retro-orbital sinus at a dose of 2×10^9 particles in a final volume of 200 μl sterile phosphate-buffered saline. Animals injected with adenovirus were studied 18 h after injection (between 0700 and 1000). Plasma glucose was also studied in a group of animals 48 h after injection of the adenovirus. Animals were anesthetized with halothane, and blood samples were taken by heart puncture. The tissues (thigh muscles, inguinal fat pad, liver, and whole pancreas) were then removed and frozen immediately in liquid nitrogen. Diabetic mice injected with insulin received an intraperitoneal injection of 1.5 units of insulin (Insulatar NPH; Novo Nordisk). They were studied 2 h after the injection, following the same procedure described above.

Tissue and plasma biochemical analysis. Liver and plasma triglyceride content was determined using the Infinity triglyceride reagent kit (Sigma). Liver glycogen content was determined according to Bergmeyer (17). Briefly, 100 mg liver was homogenized in 0.2 mol/l acetate buffer and incubated in the presence of α-(1–4),(1–6)-amyloglucosidase (Roche, Meylan, France) for 1 h at 55°C. Glucose and β-hydroxybutyrate concentrations were measured by enzymatic spectrophotometric assays.

Enzyme activities. GK activity was assessed in liver extracts by an enzymatic method that measures the production of NADPH from NADP in the presence of glucose-6-phosphate deshydrogenase from yeast (Sigma). The assay was performed at 0.5 and 100 mmol/l glucose at 25°C, and glucokinase activity was taken as the difference between the reaction rates at the two glucose concentrations and was expressed in milliunits per milligram of protein. The activity of PEPCK was determined using the NaH [^{14}C] CO_3 fixation assay described by Chang et al. (18). The activity of FAS was determined by the spectrophotometric assay described by Linn (19).

Isolation of total RNA and Northern blot hybridization. Total cellular RNAs were extracted from whole liver using the guanidine thiocyanate method (20) and prepared for Northern blot hybridization as previously described (21). Labeling of each DNA probe with [α-^{32}P]dCTP was performed by random priming (Rediprime labeling kit; Amersham, Buckinghamshire, U.K). FAS, GK, PEPCK, SREBP-1c, and S14 cDNAs were obtained as previously described (9). Mouse LDL receptor cDNA was obtained by reverse transcriptase–polymerase chain reaction using the following primers: sense, 5′-GAGGAACTGGCGGCTGAA-3′; anti-sense, 5′-GTGCTGGATGGGGAGGT CT-3′. Northern blots were hybridized with a ribosomal 18S probe to verify that equivalent amounts of total RNA were loaded in each lane.

Immunoblotting. Nuclear extracts of mouse liver were prepared according to Gorski et al. (22). A volume of 30 μg nuclear extract was mixed with SDS loading buffer and subjected to SDS/PAGE on a 12% acrylamide gel. Proteins were electrotransferred on to a Hybond C nitrocellulose membrane (Amersham Pharmacia Biotech). Recombinant SREBP-1c was detected with a mouse monoclonal antibody (IgG-2A4) raised against amino acids 301–407 of human SREBP-1. Primary antibodies against SREBP-1c were used at 4 mg/ml, and detection of signals was performed using an enhanced chemiluminescence Western blot detection kit (Amersham Pharmacia Biotech) with anti-mouse horseradish peroxidase–conjugated IgG as second antibody.

Statistical analysis. Results are expressed as the means ± SE. Statistical analysis was performed with Student's t test for unpaired data. Differences were considered significant at $P < 0.05$.

RESULTS

Establishment of diabetes in CBA mice. After STZ injection, mice were checked daily for glycosuria and plasma glucose concentration. Once their hyperglycemia was stable (i.e., after ~1 week), mice were divided into two groups of similar hyperglycemia: 470 ± 50 mg/dl (n = 15) for the group injected with null adenovirus and 460 ± 60 mg/dl (n = 15) for the group injected with SREBP-1c adenovirus. The weight of nondiabetic control mice was 28.5 ± 0.7 g (n = 5), and the weight of mice 1 week after the onset of diabetes was 22.5 g ± 0.2 (n = 16). Diabetic mice were hyperketonemic, as shown by β-hydroxybutyrate measurements of 0.447 ± 25 mmol/l (n = 8) vs. 0.095 ± 4 mmol/l (n = 5) in nondiabetic mice.

At 48 h after the injection with null adenovirus or SREBP-1c adenovirus, we found that in the two groups (n = 8 in each group), body weight (22.2 ± 0.2 and 23 ± 0.5 g, respectively) and liver weight (1.1 ± 0.1 and 1.30 ± 0.06 g) were similar.

Tissue expression of recombinant SREBP-1c mRNA and protein. Previous studies in rodents indicated that systemic infusion of recombinant adenovirus results in a preferential targeting of the transgene to the liver (23–25). To verify that the expression of recombinant SREBP-1c was indeed restricted to the liver, we performed Northern blots with total RNA extracted from different tissues of mice injected with the null or SREBP-1c adenovirus (Fig. 1*A*). Injection of the SREBP-1c adenovirus resulted in a marked expression of the transgene in the liver, whereas its expression remained undetectable in other tissues (Fig. 1*A*), even after overexposure of the blots (not shown). Nuclear extracts from the livers of SREBP-1c adenovirus–injected mice showed high amounts of recombinant SREBP-1c (Fig. 1*B*). As previously described in diabetic rats (5), endogenous SREBP-1c was not detected in nuclear extracts from STZ-induced diabetic mice.

Effect of adenovirus-mediated expression of SREBP-1c on hepatic gene expression and enzyme activities: comparison with insulin injection. Insulin injection increased the mRNA content of endogenous SREBP-1c in diabetic mice (Fig. 2), as shown previously in diabetic rats (5) and in agreement with in vitro studies in cultured hepatocytes (4). As expected, insulin increased the hepatic expression of known insulin-inducible genes, GK, FAS, and S14. The expression of the LDL receptor was also increased by insulin treatment, as described previously in

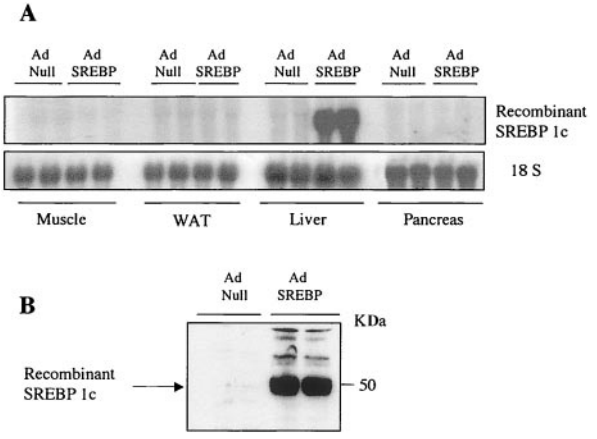

FIG. 1. Analysis of SREBP-1c mRNA and protein in various tissues of injected diabetic mice. CBA diabetic mice were injected with 2×10^9 pfu of null adenovirus (Ad Null) or SREBP-1c adenovirus (Ad SREBP) and studied 18 h later. *A*: Liver, white adipose tissue (WAT), muscle, and pancreas were quickly removed and frozen at −80°C. Total RNA was extracted. The transgene mRNA (1.3 kDa) was detected on a Northern blot hybridized with a cDNA encoding SREBP-1. To verify that equivalent amounts of total RNA were loaded in each lane, the blot was hybridized with a ribosomal 18S probe. *B*: Livers of mice infused with null adenovirus or SREBP-1c adenovirus were homogenized rapidly for preparation of nuclear extracts, and a Western blot was performed as described in RESEARCH DESIGN AND METHODS. The blots are representative of two different experiments, i.e., of four mice per group.

diabetic rats injected with insulin (5). Finally, insulin repressed the expression of PEPCK (Fig. 2*A*).

Forced expression of the mature form of SREBP-1c in the liver of diabetic mice markedly stimulated the expression of GK, lipogenesis-related proteins, FAS, and S14 as well as the LDL receptor (Fig. 2*B*). Surprisingly, it also led to a marked decrease in the expression of PEPCK, which was then nearly undetectable at the mRNA level (Fig. 2*B*). Thus, the presence of an active form of SREBP-1c in the liver mimics both inductive and repressive effects of insulin and results in a pattern of hepatic gene expression nearly identical to that of insulin injection.

Functional consequences of adenovirus-mediated expression of SREBP-1c on hepatic metabolism and glucose homeostasis. The consequences of SREBP-1c overexpression at the gene level were also apparent when enzyme activities were measured. GK and FAS activities were increased 1.8- and 2.1-fold, respectively (Table 1), whereas PEPCK activity was decreased nearly fourfold. In the liver, this led to a fourfold increase in liver glycogen concentration and a doubling of triglyceride concentration (Fig. 3), clearly underlying the increased capacity for glucose utilization in this organ. In contrast, the circulating concentration of triglycerides was decreased (Fig. 3). A striking feature of SREBP-1c hepatic overexpression was the marked decrease in the blood glucose of diabetic mice. Indeed, their blood glucose was already lower after 18 h when compared with diabetic mice injected with the null adenovirus (not shown) and nearly normalized 48 h after the injection, whereas the glycemia of diabetic mice injected with the null adenovirus remained high (Fig. 3).

DISCUSSION

SREBP-1c, a key factor of the regulation of glucose homeostasis. The transcription factors of the SREBP family SREBP-2, -1a, and -1c have been involved in the control of the expression of genes related to the metabolism of specific nutrients. The comprehensive work of Brown and Goldstein (26) has demonstrated the involvement of SREBP-2 and -1a in the control of genes involved in cholesterol synthesis and uptake. In vitro and in vivo studies on SREBP-1c first related this isoform to the upregulation of genes of the lipogenic pathway (9,27–29), a condition encountered during high carbohydrate intake.

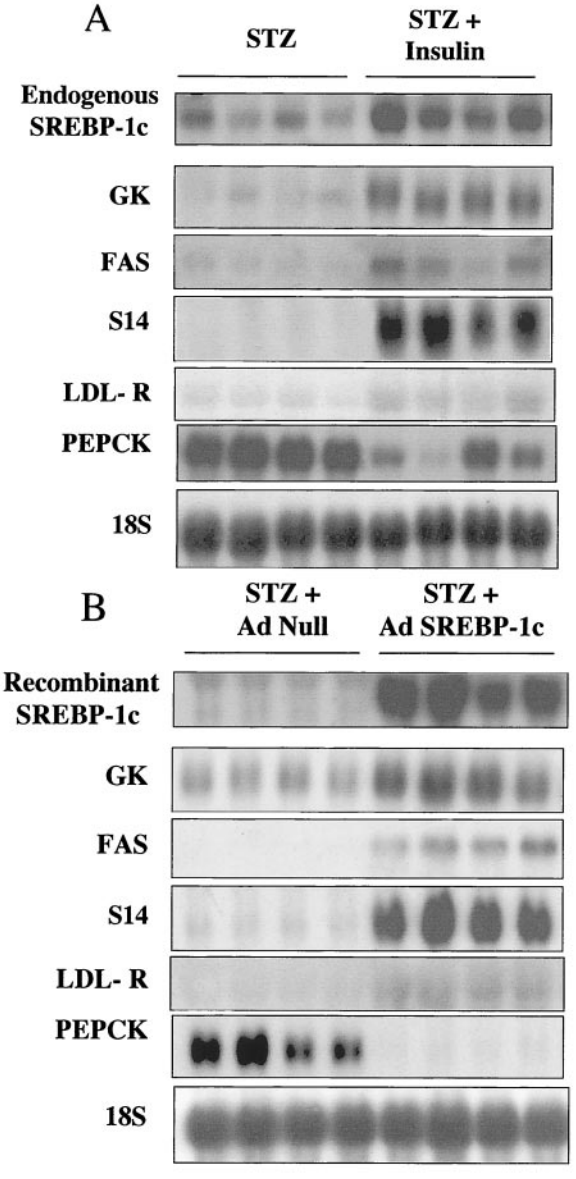

FIG. 2. Hepatic gene expression in diabetic mice injected with null adenovirus (Ad Null) or SREBP-1c adenovirus (Ad SREBP-1c) and comparison with hepatic gene expression of diabetic mice injected with insulin. *A*: Diabetic mice were injected with 1.5 units of insulin and studied 2 h after the injection. Total RNAs were extracted and prepared for Northern blot hybridization. A Northern blot hybridized with FAS, GK, PEPCK, SREBP-1, and S14 probes and representative of two different experiments is shown. The Northern blot was hybridized with a ribosomal 18S probe to verify that equivalent amounts of total RNA were loaded in each lane. *B*: Diabetic mice were injected with 2×10^9 pfu of null adenovirus or SREBP-1c adenovirus and studied 18 h later. Total RNA was extracted and prepared for Northern blot hybridization as described in RESEARCH DESIGN AND METHODS. A Northern blot hybridized with FAS, GK, LDL receptor, PEPCK, SREBP-1, and S14 probes and representative of two independent experiments is shown. The Northern blot was hybridized with a ribosomal 18S probe to verify that equivalent amounts of total RNA were loaded in each lane.

TABLE 1
Diabetic mice injected with 2×10^9 pfu of null adenovirus or SREBP-1c adenovirus and studied 18 h later

Enzyme activity	STZ + adenovirus null	STZ + adenovirus SREBP-1c
n	8	9
Glucokinase	20.6 ± 0.7	36.2 ± 1.0*
FAS	7.9 ± 0.7	16.9 ± 1.0*
PEPCK	12.6 ± 1.5	3.4 ± 0.2*

Data are means ± SD (in milliunits per milligrams protein). Liver was quickly removed and frozen at −80°C for subsequent enzyme activity determination. *Difference statistically significant for $P < 0.001$.

Later, it was found that SREBP-1c transcriptional activity could be regulated by insulin at the gene level (4,5,8) and possibly by post-translational mechanisms (30), thus bridging insulin signaling and the well-known ability of this hormone to enhance long-term lipogenic capacity.

Finally, the discovery that GK, a key enzyme of hepatic glucose utilization regulated by insulin at the gene level, was a target of SREBP-1c (9) has extended the potential importance of this factor in the control of glucose metabolism. This study represents the first evidence that SREBP-1c indeed has a pivotal role in vivo in glucose homeostasis by transducing the effects of insulin on hepatic gene expression.

Hypoglycemic effects of SREBP-1c hepatic overexpression. What are the mechanisms that can explain the strong hypoglycemic effects of SREBP-1c? Induction of GK by SREBP-1c is likely to have an important role in the hypoglycemic action of this transcription factor. Indeed, in transgenic diabetic mice overexpressing GK in the liver (15), in transgenic mice with additional GK gene copy (31), and in rats injected with a recombinant adenovirus expressing GK (24), blood glucose was decreased. The glycemia of diabetic mice was even nearly normalized by hepatic GK overexpression (15). Increased GK activity can in turn enhance hepatic glucose flux into glycogen synthesis (15,24,31) (Fig. 3) and in the lipogenic pathway, as shown by the increased hepatic triglyceride content (24) (Fig. 3). This latter event would be favored by the concomitant enhanced expression of lipogenic enzymes such as FAS because their gene expression is sensitive to both insulin and increased glucose metabolism (32). Interestingly enough, and in contrast with previous findings in rats overexpressing hepatic GK (24), the increased hepatic triglyceride content was not paralleled with an increased serum triglyceride concentration, but rather with a decreased concentration. The same kind of result was found in transgenic mice overexpressing SREBP-1c (27). This was attributed to the induction by this transcription factor of the hepatic LDL receptor, which could mediate the degradation of VLDL particles before their secretion. Indeed, when transgenic mice overexpressing SREBP-1a were mated with mice in which the LDL receptor had been disrupted, a considerable increase in plasma lipids was revealed (33). This explanation could also hold in the present study, because the expression of the LDL receptor was enhanced in the liver of diabetic mice injected with the SREBP-1c adenovirus.

In addition to decreased glucose utilization, diabetes is characterized by excessive hepatic glucose production that is linked to active gluconeogenesis. Because of a lack of insulin or insulin resistance, insulin is unable to repress the transcription of one of the key enzymes of this pathway, cytosolic PEPCK, which is regulated at the transcriptional level by this hormone. One of the striking findings of this study is that overexpressed SREBP-1c also strongly represses the expression and activity of PEPCK and thus should induce a marked decrease of endogenous glucose production. It is therefore important to assess whether this is a direct effect of SREBP-1c on the PEPCK promoter. It has been already noted that the overexpression of GK can decrease PEPCK expression in diabetic mice (15). This was attributed to an inhibitory effect of a GK-mediated increased glucose metabolism, because glucose or one of its metabolites represses PEPCK promoter activity (34,35). In the present study, because of the increased GK activity, a glucose effect could certainly contribute to the strong decrease of PEPCK expression and activity. However, *1*) the repression caused by SREBP-1c overexpression is equally or even more potent compared with that induced by insulin injection, and *2*) recent studies performed in vitro and using transfection experiments have shown that SREBP-1c is a potent repressor of PEPCK promoter activity (36). STZ-induced diabetes is characterized by a rise in glucagon concentration. It is interesting to emphasize that SREBP-1c overexpression is thus able, like insulin, to fully antagonize the effects of glucagon on PEPCK expression, as seen in this study and those of Chakravarty et al. (36) and Sasaki et al. (37).

In summary, the hypoglycemic effect of SREBP-1c, which mimics the known action of insulin, is probably the consequence of both an increase in glucose utilization and a decrease in glucose production by the liver. Given the

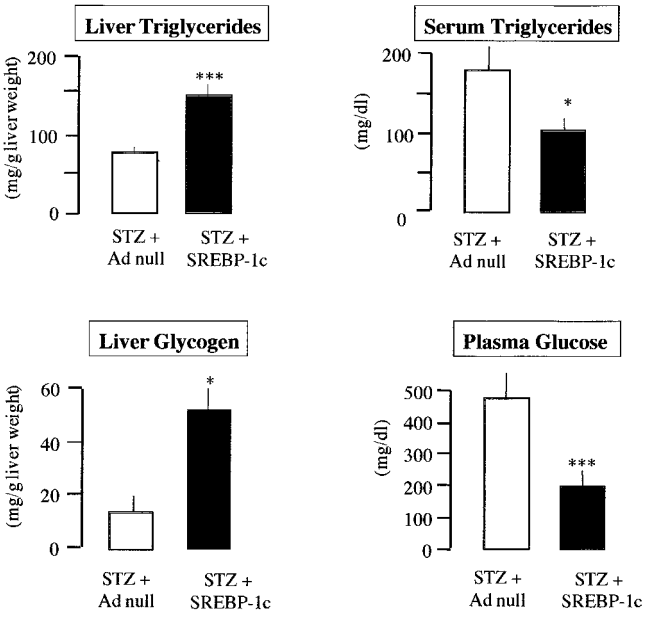

FIG. 3. Hepatic glycogen and triglyceride concentrations, plasma glucose, and serum triglycerides in diabetic mice injected with null adenovirus (Ad Null) or SREBP-1c adenovirus (Ad SREBP-1c). Diabetic mice were injected with 2×10^9 pfu of null adenovirus or SREBP-1c adenovirus and studied 18 h later for all parameters shown here except plasma glucose, which was analyzed 48 h after adenovirus injection. The results are the mean ± SE of eight values in each group. *Difference statistically significant for $P < 0.05$; ***difference statistically significant for $P < 0.001$.

results of this study, it would certainly be of interest to assess, under various nutritional conditions, the glucose homeostasis of existing models of either mice deleted for the SREBP-1 gene (38) or transgenic mice overexpressing SREBP-1 isoforms, although in the latter case, the situation might be confounded by the fact that in order to confer hepatic specificity to the transgene, the SREBP-1 gene was driven by a PEPCK promoter (27,39).

In conclusion, peroxisome proliferator–actiavted receptor-γ was the first transcription factor involved (by a still unknown mechanism) in the control of insulin sensitivity and was used as a therapeutic target in type 2 diabetes. Our studies point to another transcription factor, SREBP-1c, as *1*) having a major role in the long-term control of glucose homeostasis by insulin; *2*) being a potential causal factor in a number of diseases related to insulin resistance and blood glucose disturbances, such as obesity, syndrome X, and type 2 diabetes; and *3*) being a potential therapeutic target in these diseases.

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