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# Metabolic dysregulation in the SHROB rat reflects abnormal expression of transcription factors and enzymes that regulate carbohydrate metabolism

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**Abstract**

The Koletsky (SHROB) strain of rats is spontaneously hypertensive and displays insulin resistance, hyperglucagonemia and hypertriglyceridemia but is normoglycemic under fasting conditions. The aim of this study was to unravel the pattern of expression of genes encoding key regulatory enzymes involved in carbohydrate metabolism in the liver and kidney that may be impacted in this strain. We found that SHROB animals have decreased β-adrenergic receptor density and, consequently, blunted increases in cAMP levels in response to β-adrenergic agonists. They also have lower levels of hepatic as well as renal phospho*enol*pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) mRNA and protein than their lean littermates. Expression of the genes for glycogen phosphorylase and glycogen synthase was also decreased. Hepatocytes from the SHROB animals exhibited glycogen depletion of only 50% compared to 86% by hepatocytes from lean littermates when challenged with either glucagon or forskolin to stimulate adenylyl cyclase. The expression of C/EBPα and C/EBPβ, two key transcription factors that are essential for the coordinated expression of genes involved in glucose homeostasis, was depressed in livers of the SHROB rats, as were levels of HNF-4α, PPARα and PGC-1α. We conclude that overproduction of glucose is prevented in the SHROB rats by decreased expression of the genes for glycogen phosphorylase and the gluconeogenic enzymes PEPCK and G6Pase, which may prevent progression to diabetes in this model.


*Keywords:* Koletsky rats; Metabolic syndrome; Carbohydrate metabolism; Gene expression; Hypertension; β-Adrenergic receptor

## 1. Introduction

Metabolic syndrome X is a cluster of abnormalities characterized by insulin resistance, hypertension, hypertriglyceridemia and obesity [1]. Studies in both human and animals strongly suggest a relationship between these metabolic abnormalities and the development of diabetes and cardiovascular disease [2]. The Koletsky strain of rats, a genetically obese spontaneously hypertensive rat (SHROB/Kol), is a useful animal model for investigating this relationship because this strain exhibits most of the clinical signs of metabolic syndrome X [3]. The SHROB obese genotype, designated $fa^k$, is recessive and involves mutation of the gene for the leptin receptor; this mutation results in a premature stop codon that impacts the extracellular domain of the leptin receptor [3]. Lean siblings of the SHROB, carrying one or no $fa^k$ allele, are spontaneously hypertensive (SHR) but exhibit only mild insulin resistance relative to normotensive rats [4]. The SHROB carries two $fa^k$ alleles, is incapable of central and peripheral responses to leptin [5,6] (leptin resistant) and has circulating leptin levels that are nearly 170-fold higher than its lean siblings [3]. SHROB rats are hyperlipidemic, hyperinsulinemic and normoglycemic and have an abnormal response to a glucose load compared to their lean counterparts [3]; they also have significantly higher fasting plasma glucagon concentrations and insulin/glucagon molar ratios than lean SHR littermates [7].

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Conceptually, the imbalance between insulin and glucagon in the SHROB animal could lead to dysregulation of genes encoding hepatic enzymes that regulate glucose homeostasis. In this study, we have investigated whether the metabolic manifestations in the obese Koletsky rats impact expression of genes coding for key enzymes involved in carbohydrate metabolism in the liver. We found that expression of the genes for phospho*enol*pyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), glycogen synthase and glycogen phosphorylase was dramatically decreased in the liver of the SHROB rat. Glucagon and forskolin failed to stimulate glycogenolysis in the SHROB rat but stimulated this process in the SHR rats. We hypothesize that this metabolic pattern could explain the inability of the SHROB rat to mobilize hepatic glycogen.

## 2. Materials and methods

### 2.1. Animals

#### 2.1.1. SHR and SHROB rats

Homozygous female SHROB ($fa^k/fa^k$; weight: 590±20 g, 30 weeks old) and age- and sex-matched lean SHR littermates ($Fa^k/fa^k$ or $Fa^k/Fa^k$; weight: 281±9 g) were used in all experiments. The animals were housed individually and were provided food (Tek lab formula 8664) and water ad libitum. SHR and SHROB rats were fasted for 12 h; they were then anesthetized with isoflurane by inhalation, and the liver and kidney were freeze-clamped and stored at −80°C pending analysis.

#### 2.1.2. Isolation of hepatocytes

Hepatocytes were isolated from homozygous female SHROB ($fa^k/fa^k$) and age- and sex-matched lean SHR littermates ($Fa^k/fa^k$ or $Fa^k/Fa^k$) after an overnight fast and used in primary cultures as previously described [8].

#### 2.1.3. Isolation of RNA and Northern blotting analysis

Primary hepatocytes ($10^6$) were plated onto 100-mm plates and treated as described in the legends to figures. RNA was extracted from tissues and hepatocytes, using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Northern blotting analysis was performed as previously described [9].

#### 2.1.4. Leptin knockout mice

Ten-week-old wild-type and leptin knockout mice were fed a regular mice chow diet. Following a 12-h fast, the animals were anesthetized; the livers were freeze-clamped and stored at −80°C pending analysis.

#### 2.1.5. Assay of metabolites

Cell monolayers were scraped into 30% KOH to measure glycogen content. The extract was then boiled for 15 min and centrifuged at 5000×*g* for 15 min. Glycogen was determined as glucose after incubation with amyloglucosidase [10]. Lactate concentration in the incubation medium was measured as described [9].

#### 2.1.6. Immunoblotting

About 100 mg of tissue was homogenized in 0.5 ml of lysis buffer [25 mM Tris–HCl (pH 7.4), 0.5 mM EGTA, 25 mM NaCl, 1% Nonidet P-40, 2 mM $Na_3VO_4$, 50 mM NaF, 0.2 mM leupeptin, 1× protease inhibitor mixture; Roche Molecular Diagnosis, Indianapolis, IN] and rocked for 30 min at 4°C. Detergent-insoluble material was sedimented by centrifugation at 12,000×*g* for 10 min at 4°C. Protein content was determined by the Bradford method. For immunoblotting, 20 μg of protein was subjected to SDS-polyacrylamide gel (PAGE; 10% polyacrylamide electrophoretic). After separation, proteins were transferred to Immobilon-P membranes, and the membranes were incubated for 1 h at 4°C in Tris-buffered saline–Tween-20 containing 5% nonfat dried milk, pH 7.4. After the blocking step, membranes were washed in phosphate-buffered saline–Tween-20 wash buffer (PBST, pH 7.4) and then incubated overnight with antibody at an appropriate dilution. After washing the membranes with PBST, they were incubated with horseradish-peroxidase-conjugated secondary antibody for 1 h. The membranes were then washed several times with PBST and developed with the use of enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). Antibodies against HNF-4α, PPARα, C/EBPα and C/EBPβ were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). GSK-3α and peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α) antibodies were from Millipore (Temecula, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies were from Ambion (Austin, TX), and conjugated secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA).

#### 2.1.7. Binding to the β-adrenergic receptor (β-AR)

β-AR binding assays were carried out as described previously for porcine heart [11] to determine the density ($B_{max}$) and binding affinity ($K_d$). Briefly, rat liver samples previously frozen in liquid nitrogen were slowly thawed, minced and homogenized in 20 volumes of ice-cold HEPES-buffered isotonic sucrose (pH brought to 7.4 with Tris base) containing 1× protease inhibitor mixture (Roche Molecular Diagnosis) using a polytron homogenizer (maximum setting for 15 s). Homogenates were centrifuged at 1000×*g* for 5 min at 4°C to remove nuclei and debris. The pellet (P1) was resuspended in 20 ml of homogenization buffer and centrifuged again at 1000×*g* for 5 min. The supernatant fractions were combined and centrifuged at 48,000×*g* for 18 min at 4°C. The resulting P2 pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.7) containing 5 mM EDTA. After recentrifugation, the resulting membrane pellet was flash-frozen and stored at −70°C until used.

Radioligand binding assays with [$^{125}$I]-pindolol for determination of specific binding to β-AR sites were conducted in a total volume of 250 μl consisting of 125 μl membrane suspension (1 mg protein/ml in 50 mM Tris–HCl buffer, pH 7.7), 25 μl radioligand (0–1.0 nM) and 100 μM (−)-isoproterenol to define nonspecific binding or 0.1%

Table 1
Glycogen content in isolated hepatocytes from SHR and SHROB rats

| | SHR | | SHROB | |
|---|---|---|---|---|
| | μg/$10^6$ cells | % change | μg/$10^6$ cells | % change |
| Control | 103.0±17.8 | – | 160.5±4.8 | – |
| Glucagon | 13.8±6.9 | 86.6 | 78.5±1.8 | 51.1 |
| Forskolin | 12.3±1.2 | 88.1 | 81.9±1.0 | 49.0 |

Hepatocytes were incubated in the absence (control) or presence of 100 nM glucagon or 10 μM forskolin for 60 min. Glycogen was measured as described in Section 2. Values are expressed as means±S.E.M. of three independent experiments (except for those under the "% change" column).

ascorbic acid vehicle. The reactions were started by adding the membrane samples and were carried out for 60 min at 25°C. Incubations were terminated by vacuum filtration over glass fiber filters using a cell harvester. The filters were washed four times, each time with 5 ml of ice-cold 50 mM Tris–HCl buffer, pH 7.7, and the radioactivity was measured with a gamma counter. Radioligand binding data were analyzed by nonlinear curve fitting to a logistic equation with GraphPad Prism.

*2.1.8. cAMP assays*

Hepatocytes were incubated for 10 min with vehicle, 10 μM forskolin, 100 μM isoproterenol, 100 μM 3-isobutyl-1-methylxanthine (IBMX) or both isoproterenol and IBMX. After the incubation, cells were harvested and lysed with 1.0 ml of ice-cold 1 N HCl. The lysates were then centrifuged at 13,000×*g* for 10 min, and the supernatants were transferred to new tubes and stored at −70°C pending analysis. For the cAMP assay, samples (300 μl) were acetylated with a 1:2 mixture of acetic anhydride and triethylamine. The cAMP content was determined by using a low-pH ELISA kit (R&D Systems, Minneapolis, MN). Absorbance was read at 405 nm, using a plate reader (Tecan Rainbow, Durham, NC). Protein concentrations were determined by the bicinchoninic acid method [12].

## 3. Results

### *3.1. Metabolic characteristics of the SHROB rats*

We have previously reported the metabolic profile of the SHR and SHROB rats; SHROB rats have significantly higher plasma insulin, glucagon and FFA concentrations compared with lean littermates [7]. Importantly, they display no significant elevation in fasting blood glucose relative to lean SHR. Furthermore, liver biopsies of SHROB rats contain high glycogen content, especially after an overnight fast [3]. To investigate potential mechanism(s) underlying this metabolic manifestation, we first studied the effects of glucagon and forskolin in isolated hepatocytes from fasted SHROB and SHR rats, using concentrations that are most likely to induce high levels of glycogen breakdown. As shown in Table 1, the treatment with either glucagon or forskolin was sufficient to deplete hepatic glycogen in the SHR by at least 86%; in contrast, the treatment resulted in only about 50% depletion in the SHROB rats. Although the net quantity of glycogen mobilized (85–89 μg glucose/$10^6$ cells) was similar in SHR and SHROB hepatocytes, the amount of residual glycogen was much higher in SHROB hepatocytes (~80 μg glucose/$10^6$ cells) than in SHR hepatocytes (~14 μg glucose/$10^6$ cells), suggesting resistance to glycogen depletion in hepatocytes from SHROB animals. The ability of both cell populations to metabolize glucose to lactate was not substantially different and was not substantially altered by treatment with glucagon, insulin or forskolin (Fig. 1).

The diminished ability of hepatocytes from SHROB rats to respond to cAMP-elevating agents, with respect to glycogen depletion, may be due to defect(s) in cell-surface receptors or to potential defect(s) in post-receptor signaling. Since increase in intracellular cAMP levels in the liver can be induced by either glucagon or β-adrenergic agonists, we analyzed β-adrenergic receptor binding in isolated plasma membranes from livers of SROB rats, using the high-affinity β-adrenergic antagonist [$^{125}$I]-iodopindolol as the radioligand. This approach indicated a decrease in the number of β-ARs in liver membranes of SHROB rats compared to the SHR littermates. The $B_{max}$ (mean±S.E.M.) in SHROB was 550±84 fmol/mg protein, relative to 893±123 fmol/mg protein in hepatic membranes isolated from SHR ($P<.05$); the binding affinity, measured as $K_d$, did not differ in the two types of membranes (114±49 pM in SHROB and 76±39 pM in SHR; Fig. 2A).

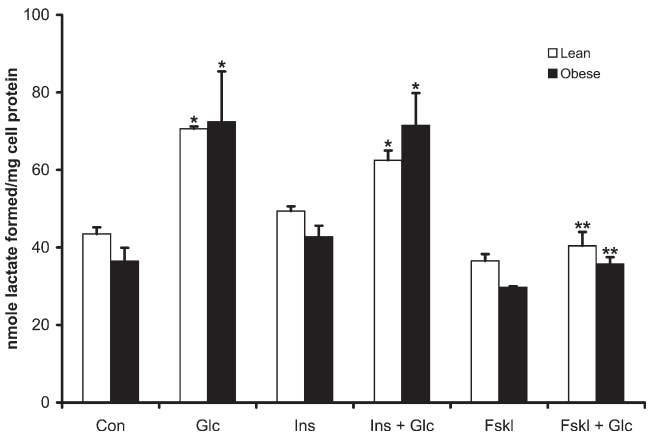


Fig. 1. Lactate formation in isolated hepatocytes in response to various treatments. Freshly isolated rat hepatocytes were cultured in RPMI 1640 medium for 16 h. The medium was then replaced with basal medium consisting of RPMI-1640 medium containing 5 mM glucose. The cells were then left untreated (Con) or treated for 30 min with 20 mM glucose (Glc), 10 nM insulin (Ins), 10 nM insulin and 20 mM glucose (Ins+Glc), 10 μM forskolin (Fskl) or forskolin and 20 mM glucose (Fskl+Glc). The lactate content in the medium was measured and is expressed as nanomoles of lactate formed in 30 min/mg cell protein. The data are the means±S.E. of three different experiments. The significance between the means of two groups was assessed by using Student's *t* tests. *Statistical differences ($P<.05$) between Con and Glc or Glc+Ins. **Statistical differences between Glc and Fskl and Fskl+Glc.

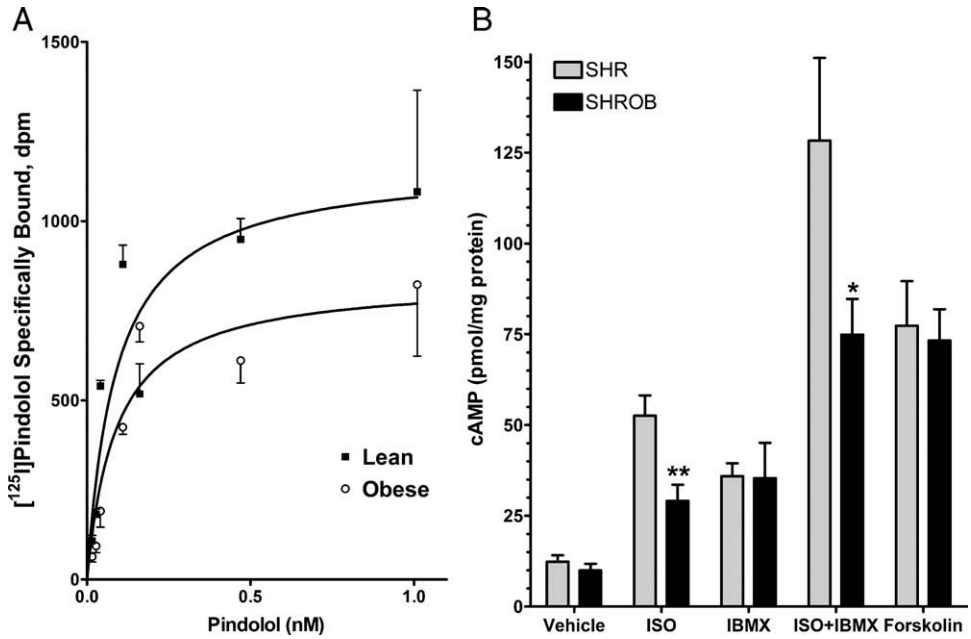


Fig. 2. β-AR receptor binding and cAMP levels in the liver of SHR and SHROB rats. In Panel A, radioligand binding assays with [$^{125}$I]-pindolol for determination of specific binding to β-AR sites were performed as described in Section 2. The curve is the best fit to a hyperbolic function with $B_{max}$ as the asymptote. In Panel B, hepatocytes were incubated in RPMI-1640 medium and treated for 10 min with vehicle, 100 μM isoproterenol, 100 μM IBMX or 10 μM forskolin, and cAMP levels were measured. The assay was stopped with 1.0 ml ice-cold 1 N HCl; the cAMP content in the medium was determined using ELISA. Newman–Keuls test was used to determine statistical differences ($P<.05$).

To test whether the SHROB rats may possess post-receptor defect(s), we monitored cAMP production in isolated hepatocytes, in response to isoproterenol, forskolin or IBMX, an inhibitor of cAMP degradation by phosphodiesterases (PDEs). In response to either IBMX or forskolin, there were no differences in cAMP levels in hepatocytes from SHR and SHROB rats (Fig. 2B). The similar response to forskolin in hepatocytes from both groups of animals rules out a defect in the capacity of adenylyl cyclase to form cAMP in the SHROB animals. In Western blots of liver biopsies from SHR and SHROB rats, we found no differences in the expression of the main isoforms of PDE (PDE3A and PDE3B) that degrade cAMP in hepatocytes, adipocytes and pancreatic β-cells [13–15] (results not shown), suggesting no apparent defect in the capacity to degrade cAMP. Strikingly, isoproterenol-stimulated cAMP production in the SHROB rats was decreased by 49% compared to the SHR rat (Fig. 2B). Although the addition of isoproterenol and IBMX together increased cAMP levels in hepatocytes from both SHR and SHROB hepatocytes, the magnitude of the response was much lower in hepatocytes from SHROB rats than in those from SHR rats, indicating that the β-adrenergic signaling pathway is defective in the liver of SHROB animals. This conclusion is consistent with the reduced receptor density shown in Fig. 2A, although additional defect in Gs function cannot be ruled out.

The expression of a number of hepatic genes is regulated by cAMP signaling. Since SHROB animals exhibit decreased numbers of hepatic β-ARs, lowered production of cAMP levels in the liver of SHROB rats could be expected to result from decreased response to

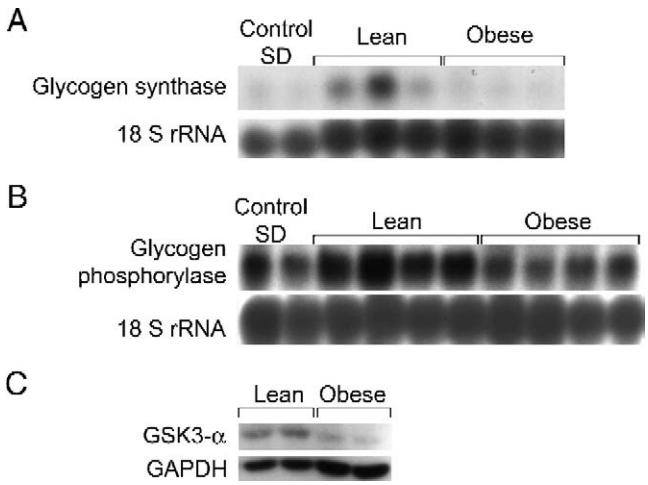


Fig. 3. Glycogen phosphorylase and glycogen synthase mRNA levels in the liver of SHR and SHROB rats. Total RNA was isolated from liver biopsies of SHR and SHROB rats, and mRNA levels for glycogen synthase (A) and glycogen phosphorylase (B) were measured by Northern blot analysis. The same blots were reprobed for 18S rRNA as loading control. (C) GSK3α in the liver of SHR and SHROB rats. About 100 mg of liver was homogenized in lysis buffer as indicated in Section 2. Twenty micrograms of protein in this homogenate was separated onto a 7.5% SDS-PAGE and analyzed by Western blotting. The same blot was reprobed for GAPDH as loading control. Each lane corresponds to an individual animal.

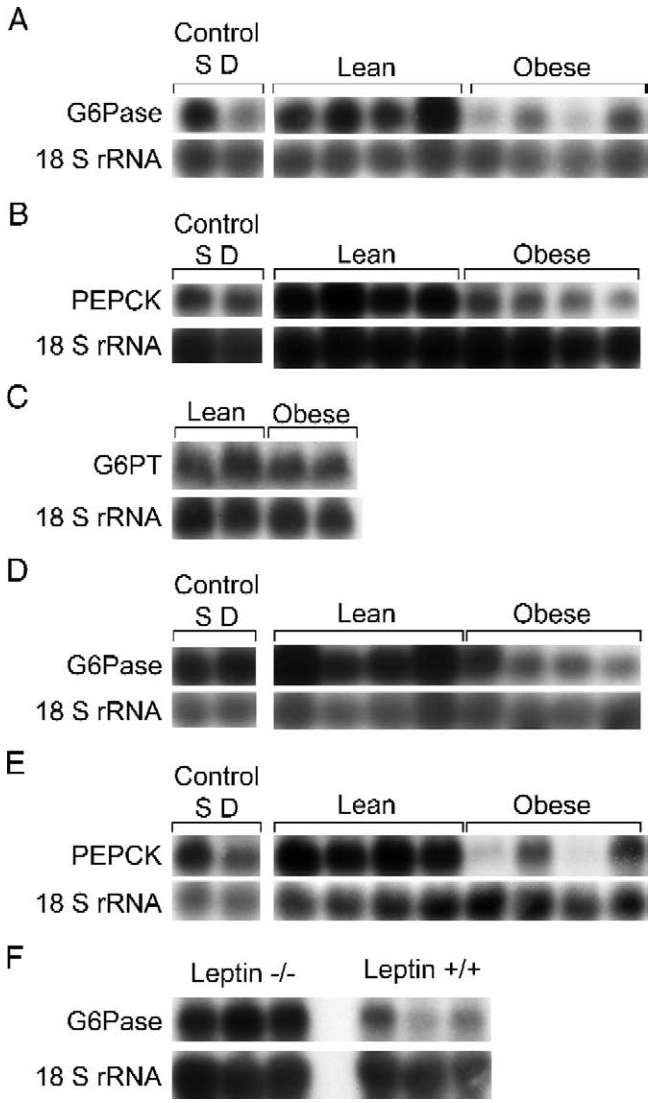


Fig. 4. G6Pase, PEPCK and G6PT mRNA levels in the liver and kidney of SHR and SHROB rats. Total RNA was isolated from liver biopsies of SHR and SHROB rats and of leptin −/− and +/+ mice, and mRNA levels were assessed by Northern blot analysis. Panels show mRNA levels for G6Pase (A), PEPCK (B) and G6PT (C). G6Pase (D) and PEPCK (E) mRNA levels in the kidney of SHR and SHROB rats. (F) G6Pase mRNA levels in the liver of leptin −/− and +/+ mice. In all cases, the same blots were reprobed for 18S rRNA as loading control. Representative Northern blots are shown. Each lane corresponds to an individual animal.

hormones (such as catecholamines) that increase cAMP production. Such defect could have long-term effects on metabolic characteristics of the SHROB rat. For this reason, we measured the mRNA levels for glycogen synthase and glycogen phosphorylase, two enzymes that regulate overall glycogen metabolism. Although there was animal-to-animal variation as would be expected, the overall results reveal that the mRNA level for glycogen synthase was much lower in the liver of SHROB animals than in the liver of SHR littermates (Fig. 3A). Interestingly, the protein level of glycogen synthase kinase 3α (an enzyme known to inactivate glycogen synthase) was also much lower in the liver of SHROB rats (Fig. 3C) than in the liver of SHR animals. The mRNA level for glycogen phosphorylase was similar in the liver of SHR rats compared to normal Sprague–Dawley rats but was two- to threefold lower in the liver of the SHROB rats (Fig. 3B). These results may reflect a different glycogen turnover rate between SHR and SHROB rats.

### 3.2. Concurrent regulation of genes encoding gluconeogenic enzymes in SHROB rats

To investigate further the mechanism by which the SHROB animals maintain glucose homeostasis in the fasted state, we studied the expression of genes coding for key gluconeogenic enzymes, that is, PEPCK and G6Pase, because their expression is known to be up-regulated by agents that elevate cellular cAMP levels [16,17]. We found that the mRNA level for G6Pase was approximately fivefold lower in the liver of SHROB rats than in the liver of age-matched SHR and Sprague–Dawley rats (Fig. 4A). The change in PEPCK mRNA level (Fig. 4B) was similar to that of G6Pase. The mRNA level for the glucose-6-phosphate translocase (G6PT), which is associated with the G6Pase complex, was also lower in the liver of the obese (SHROB) animals compared to SHR rats (Fig. 4C). As both G6Pase and PEPCK are expressed in the kidney, we also investigated whether the decrease in the expression of these two genes was also observable in the kidney. Fig. 4 shows that both G6Pase (Panel D) and PEPCK (Panel E) mRNA levels were also decreased in the kidneys of the SHROB compared to the SHR rats, indicating their generalized decrease in these gluconeogenic tissues.

The chronically elevated insulin concentration in the Koletsky strain would be expected to down-regulate genes that are negatively regulated by insulin such as the genes for PEPCK and G6Pase. Using the G6Pase gene as a test gene, we studied whether hyperinsulinemia may have contributed to the lowered expression of such genes. For these experiments, we isolated hepatocytes from SHR and SHROB rats, and after the attachment period, the hepatocytes were cultured overnight in insulin-free media. Cells were then treated with or without insulin for 4 h before isolating total RNA for Northern blot analysis. This treatment decreased, to the same extent, G6Pase mRNA level in hepatocytes isolated from both SHR and SHROB rats (results not shown), suggesting that the liver of the SHROB rat remains sensitive to the action of insulin and is not differentially sensitized to the hyperinsulinemia in this animal.

Because the SHROB rat is leptin resistant, we wondered whether the reduced expression of the glucose-metabolizing enzymes could be related to lack of leptin action in these animals. To explore this idea, we used the leptin knockout mice, which is a good model of defective leptin function. Again, using the G6Pase gene as a representative gene, we

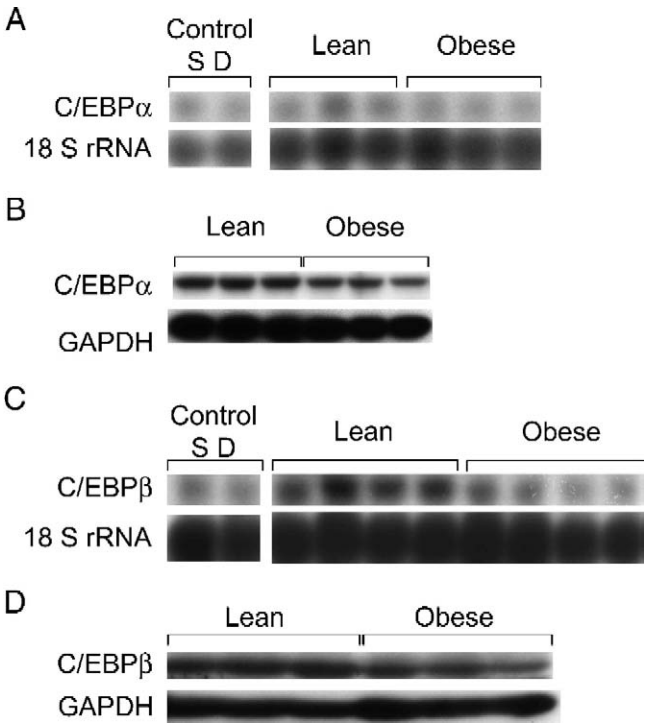


Fig. 5. Levels of C/EBPα and C/EBPβ in the liver of SHR and SHROB rats. Total RNA was isolated from liver biopsies of SHR and SHROB rats, and mRNA levels for C/EBPα (A) and C/EBPβ (C) were measured by Northern blot analysis. The same blot was reprobed for 18S rRNA as loading control. (B and D) C/EBPα and C/EBPβ protein levels in the liver of SHR and SHROB rats determined by Western blotting. About 100 mg of liver was homogenized in RIPA buffer as indicated in Section 2. Twenty micrograms of homogenate was separated onto a 7.5% SDS-PAGE and analyzed by Western blotting. The same blot was reprobed for GAPDH as loading control.

measured its expression in leptin compared to wild-type controls. The results showed substantially increased expression of G6Pase mRNA (Fig. 4F) in the liver of leptin knockout mouse compared to wild-type age-matched controls. These results are in direct contrast to the decrease in G6Pase mRNA level in the liver of SHROB rats, suggesting that lack of leptin signaling is not the primary cause of the dysregulation of G6Pase gene expression.

### *3.3. Expression of transcription factors that regulate transcription of key enzymes in carbohydrate metabolism is blunted in SHROB rats*

The transcription factors C/EBPα and C/EBPβ are highly expressed in the liver and have been documented to control the expression of a number of genes encoding key enzymes involved in gluconeogenesis and lipid metabolism [18]. Analyses of these transcription factors by Northern and Western blots of liver extracts from SHR and SHROB rats showed an about two-fold decrease in C/EBPα mRNA and protein levels in the SHROB rats (Fig. 5A and B); the decrease in C/EBPβ mRNA level was more dramatic (four- to five-fold decrease) than in C/EBPα mRNA (compare Fig. 5C to Fig. 5A). The decrease in C/EBPβ protein concentration was about the same (twofold) as that in C/EBPα protein (compare Fig. 5D to Fig. 5B). It has previously been established that HNF-4α, a liver-enriched transcription factor that is involved in hepatocyte differentiation, regulates ureagenesis as well as glucose and fatty acid metabolism [19,20]. We found that livers of SHROB rats contained three- to four-fold lower amounts of this transcription factor than livers of SHR littermates (Fig. 6A). PGC-1α, which is known to enhance HNF-4α-induced transcriptional activation of G6Pase and PEPCK gene promoters [21–23], was about two- to three-fold lower in the SHROB rats than in the SHR rat (Fig. 6B). We also monitored the expression levels of PPARα because this transcription factor is also known to regulate lipid as well as glucose metabolism [24,25]. As seen in Fig 6C, SHROB rats contained about four-fold lower levels of PPARα protein than their SHR littermates. On the other hand, mRNA levels of SREBP-1c, a transcription factor known to suppress the expression of G6Pase and PEPCK genes [26,27], were higher in SHROB rats than in their SHR littermates (Fig. 6D), prompting the speculation that the increased expression of SREBP-1c may contribute to

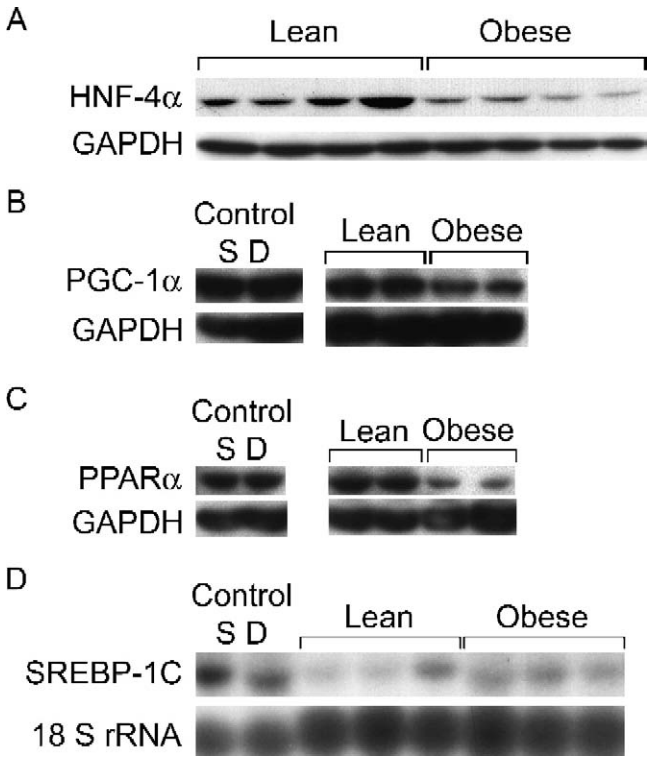


Fig. 6. HNF-4α, PGC-1α and PPARα protein levels and SREBP-1c (D) mRNA levels in the liver of SHR and SHROB rats. Livers from SHR and SHROB rats were homogenized in RIPA buffer as indicated in Section 2. Twenty micrograms of homogenate was separated onto a 7.5% SDS-PAGE and analyzed by Western blotting, using anti-HNF-4α (A), anti-PGC-1α (B) or anti-PPARα (C) antibody. The same blots were reprobed for GAPDH as loading control. (D) Total RNA was isolated from liver biopsies of SHR and SHROB rats, and mRNA was measured by Northern blot analysis. The same blot was reprobed for 18S rRNA as loading control.

the low levels of mRNAs for G6Pase and PEPCK in the liver of SHROB animals.

## 4. Discussion

SHROB rats are hyperglucagonemic and display fasting insulin levels that are 20-fold higher than their SHR littermates [3]. Despite the hyperglucagonemia, SHROB rats cannot fully mobilize their glycogen stores, suggesting a defect in glycogen turnover. Because changes in cAMP levels play an important role in the regulation of glycogen breakdown and synthesis as well as the expression of many genes that impact glucose homeostasis, we focused on enzymes involved in glycogen and glucose metabolism. Specifically, we evaluated gene expression of glycogen-metabolizing enzymes and two key gluconeogenic enzymes (i.e., G6Pase and PEPCK) as well as transcription factors that influence expression of these genes.

Both the G6Pase gene and the PEPCK gene were down-regulated in the liver as well as in the kidney of SHROB rats compared to their lean littermates. Because loss of function in liver-specific transcription factor(s) has been associated with abnormal expression of PEPCK and G6Pase in the liver [28], it was intriguing to surmise that changes in levels of transcription factors studied in this work may be causally related to the lowered expression of both PEPCK and G6Pase in SHROB rats. Since little is known about transcriptional regulation of the glycogen phosphorylase gene, it seems reasonable to speculate that decreased cAMP levels in the liver of the SHROB rat may influence expression of the gene for this enzyme, resulting in the lowered mRNA level (for this gene) observed in this study. The noted decrease in mRNA for glycogen synthase was rather surprising. However, virtually nothing is known about the molecular regulation of this gene by changes in cAMP levels at the present time; hence, it should be a fertile ground for future investigation. It would also be interesting in future studies to investigate the role for CEBPα or CEBPβ, if any, in the regulation of either glycogen synthase or glycogen phosphorylase, especially because SHROB rats exhibit decreased expression of C/EBPα and C/EBPβ concurrently with decreased expression of PEPCK and G6Pase genes.

In addition to CEBPα and CEBPβ, the levels of other transcription factors, such as HNF-4α and PPARα, as well as their common coactivator PGC-1α, which are essential for the expression of liver-specific genes, were also abnormal in the SHROB rat. PPARα, best known as a regulator of genes involved in fatty acid oxidation [29], also regulates genes involved in carbohydrate metabolism [30,31]. In addition to a role in glucose homeostasis, PPARα also seems to be involved in the control of blood pressure. Recently, PPARα was shown to be a common link in chronic steroid-induced diabetes and hypertension in mice [30]. Whereas PPARα-null mice are protected from chronic dexamethasone-induced insulin resistance and hypertension [30], reconstitution of PPARα in the liver of nondiabetic, normotensive PPARα-null mice induces glucose intolerance as well as hypertension [30]. The absence of PPARα appears to protect animals against high-fat-induced or chronically elevated glucocorticoid-induced hyperglycemia and hyperinsulinemia [30,31]. Whether the decreased PPARα expression in the SHROB rat is a protective mechanism against hypertension was not determined in this study and, thus, needs further investigation.

In our studies, we observed down-regulation of both glycogen synthase and glycogen phosphorylase in the liver of SHROB animals. Although one would expect decreased glucose cycling under these circumstances, the glycogen levels in SHROB rats remained rather high, and depletion of such glycogen with high levels of glucagon or forskolin occurred at only 50% compared to SHR animals, leaving substantial residual amounts of unhydrolyzed glycogen. It is possible that the decreased content of glycogen synthase kinase (a major enzyme that inactivates glycogen synthase) may lessen the impact of the reduction in glycogen synthase itself, leaving enough synthetic capacity that is not counterbalanced by the observed degradative capacity. Reduction in β-AR density as well as in changes in cAMP levels may act in concert with reduced expression of glycogen phosphorylase to sharply reduce the extent of glycogen mobilization. These alterations may contribute to the retention of liver glycogen during fasting.

Another relevant consideration is the potential effect of the absence of leptin receptor in SHROB rats. It should be noted that the SHROB rat lacks all isoforms of the leptin receptor, including the short form expressed in the liver [32]. Since leptin has a direct effect on the isolated liver to increase glycogen mobilization [33,34], the lack of hepatic leptin receptors might contribute to the observations in our study. Additionally, the low cAMP concentrations and reduced β-AR density may impact the expression of genes for G6Pase and PEPCK, which we noted to be decreased in the liver of SHROB rats. Normally, these genes are up-regulated by protein kinase A, downstream of cAMP production. Thus, it is possible that decreased expression of G6Pase could result in rerouting of glucose-6-phosphate toward glycogen synthesis and away from its release as glucose.

In summary, our present work provides important groundwork for future studies that will extend current understanding of the phenomenon of dysregulation of glycogen metabolism in the Koletsky strain of rats, a major model for understanding metabolic abnormalities associated with metabolic syndrome X.

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