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Transgenic mice overexpressing SREBP-1a under the control of the PEPCK promoter exhibit insulin resistance, but not diabetes

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Abstract

Sterol regulatory element-binding protein-1 (SREBP-1) is a transcription factor which regulates genes involved in the synthesis of fatty acids and triglycerides. The overexpression of nuclear SREBP-1a in transgenic mice under the control of the PEPCK promoter (TgSREBP-1a) caused a massively enlarged fatty liver and disappearance of peripheral white adipose tissue. In the current study, we estimated the impact of this lipid transcription factor on plasma glucose/insulin metabolism in vivo. TgSREBP-1a exhibited mild peripheral insulin resistance as evidenced by hyperinsulinemia both at fasting and after intravenous glucose loading, and retarded glucose reduction after insulin injection due to decreased plasma leptin levels. Intriguingly, hyperinsulinemia in TgSREBP-1a mice was markedly exacerbated in a fed state and sustained after intravenous glucose loading, and paradoxically decreased after the portal injection of glucose. TgSREBP-1a mice consistently showed very small plasma glucose increases after portal glucose loading because of a large capacity for hepatic glucose uptake. These data suggested that hepatic insulin resistance emerges postprandially. In addition, pancreatic islets from TgSREBP-1a were enlarged. These data demonstrate that SREBP-1a activation in the liver has a strong impact on plasma insulin levels, implicating the potential role of SREBPs in hepatic insulin metabolism relating to insulin resistance.
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Keywords: Transcription factor; SREBP-1a transgenic mouse; Liver steatosis; Lipodystrophy; Insulin resistance

1. Introduction

Sterol regulatory-element binding protein (SREBP) family members have been established as transcription factors regulating the transcription of genes involved in cholesterol and fatty acid synthesis. SREBP proteins are initially bound to the rough ER-membrane and form a complex with the SREBP-cleavage activating protein (SCAP), a sterol-sensing molecule. Upon sterol deprivation, SREBP is cleaved to liberate the amino-terminal portion containing a basic helix-loop-helix leucine zipper domain and enters the nucleus where it can bind to specific sterol response elements (SRE) in the promoters of target genes (reviewed in Refs. [1–3]). Three isoforms of SREBP, SREBP-1a, -1c, and -2, are known. Whereas SREBP-2 plays a crucial role in the regulation of cholesterol synthesis, SREBP-1c controls the gene expression of lipogenic enzymes (reviewed in Refs. [4–6]).

In vivo studies have demonstrated that SREBP-1 plays a crucial role in the dietary regulation of most hepatic lipogenic genes [7,8]. Physiological changes of SREBP-1 protein in normal mice after dietary manipulation such as

Abbreviations: 2-DG, 2-deoxy-glucose; HOMA-R, homeostasis model assessment on insulin resistance; ipGTT, intraperitoneal glucose tolerance tests; IRS-2, insulin receptor substrate-2; ITT, insulin tolerance tests; ivGTT, intravenous glucose tolerance tests; NEFA, non-esterified fatty acid; PEPCK, phosphoenolpyruvate carboxykinase; SCAP, SREBP-cleavage activating protein; SRE, sterol response elements; SREBP, sterol regulatory element-binding protein(s); TgSREBP-1a, overexpression of nuclear SREBP-1a in transgenic mice under the control of the PEPCK promoter; WT, wild-type (littermates)

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placement on high carbohydrate diets, polyunsaturated fatty acid-enriched diets, and fasting-refeeding regimens have also been reported [9–13]. The fuel metabolism in these nutritionally challenged mice involves a time-dependent, multi-organ, complex milieu of metabolites and hormones. To further explore the role of SREBP-1 in liver, transgenic mice that overexpress a truncated NH_2-terminal segment of human SREBP-1a, which is the constitutively active form of it, under the phosphoenolpyruvate carboxykinase (PEPCK) promoter, were created [14]. These animals exhibited massive hepatic enlargement, owing to engorgement with triglycerides and cholesteryl esters. The amounts of the mRNA for cholesterol and fatty acid biosynthetic enzymes were markedly elevated. Despite the accumulation of liver lipids, the plasma lipid levels were not elevated. An unexpected finding was a progressive involution of adipose tissue as the animals aged.

It is well established that insulin and glucose both stimulate lipogenesis. Furthermore, SREBP-1c expression is regulated by insulin and glucose in liver and adipose tissue [7,13,15–19]. Thus, SREBP-1c has been thought to be a mediator for a physiological insulin action on gene transcription. Conversely, it is well-known that the accumulation of lipids, such as increased triglyceride, content in the muscle is associated with impaired insulin sensitivity. We also recently showed that SREBP-1 directly suppressed IRS-2 and caused insulin resistance in the liver [20]. SREBP-1a transgenic mice provide us an excellent opportunity to assess the importance of this transcription factor on glucose/insulin metabolism through its direct transcription activities and/or through the resultant lipid accumulation in the liver and adipose tissue. In the current studies, glucose tolerance tests in different conditions were performed to investigate plasma glucose metabolism and to estimate the presence of insulin resistance in TgSREBP-1a mice.

2. Materials and methods

2.1. Animals

21- to 24-week-old male mice derived from breeding TgSREBP-1a [14] onto a C57BL/6 background for five generations or more were used for this study, with wild-type male littermates (WT) as controls. They were kept in 12-h light and 12-h dark cycle, and the Animal Care Committee of University of Tsukuba, following the “Principles of laboratory animal care” (NIH publication no. 85-23, revised 1985), approved animal care and procedure.

2.2. Intraperitoneal glucose test

Glucose (1.0 g/kg body wt: 4% solution) was administered by intraperitoneal injection to animals after an overnight fast or in mice fed ad libitum. Blood samples were taken at 0, 30, 60, and 120 min after glucose injection. Plasma was obtained after the centrifugation of blood samples and was stored at −20 °C until it was assayed for plasma glucose and insulin. Intraperitoneal glucose tolerance tests (ipGTT) on fasted animals were performed in TgSREBP-1a and WT, and plasma glucose levels and plasma insulin levels were measured at every time point.

2.3. Intravenous glucose tolerance tests (ivGTT)

Glucose (0.5 g/kg body wt: 20% solution) was administered intravenously to TgSREBP-1a and WT animals in a fasted or fed state. Blood samples were taken at 0, 5, 15, and 30 min after glucose injection.

2.4. Insulin tolerance test (ITT)

Human recombinant insulin (Humalin R©: Eli-Lilly, USA) (2 units/kg body wt) was injected intraperitoneally to overnight-fasted animals. Blood samples were obtained at 0, 30, 60, 90, and 120 min after insulin injection. Plasma was obtained as described above and kept for measurement of plasma glucose.

2.5. Measurement of plasma glucose and insulin levels

Plasma glucose concentrations were measured by the glucose oxidase method using a Glucose CII-test Wako© kit (Wako Pure Chemicals, Osaka, Japan). Plasma insulin concentrations were determined by ELISA using a Lebis© insulin kit (Shibayagi, Gumma, Japan) with mouse insulin as the standard.

2.6. Leptin and non-esterified fatty acid (NEFA) measurements

Blood samples taken from ad libitum fed animals were assayed to determinate the plasma levels of leptin by ELISA using commercial kit (Morinaga, Tokyo, Japan). NEFA levels were measured using the NEFA-C test Wako© kit (Wako, Osaka, Japan).

2.7. Histological analyses for pancreatic islets

Pancreases were obtained from TgSREBP-1a mice or WT controls, fixed in a solution of 4% paraformaldehyde/phosphate-buffered saline, and paraffin embedded. 5 μm sections were stained with hematoxylin–eosin. Each specimen was observed under a light microscopic and 40× images taken with a digital camera (Coolpix900©: Nikon, Tokyo, Japan). Islets were randomly selected from TgSREBP-1a and WT pancreases, and islet versus non-islet areas were quantified by measuring the numbers of pixels using Adobe Photoshop© software package (Adobe Systems, CA, USA).

2.8. Statistical analysis

Statistical significance was evaluated by two-tailed Welch's *t*-test using Microsoft Excel© software package (Microsoft, CA, USA) with Statcel© add-in program package (OMS publishing, Saitama, Japan). Differences with $P<0.05$ were deemed significant. All of the values of the results from the measurements in this study are indicated as mean±S.E.

3. Results and discussion

SREBP-1a transgenic mice, under the control of PEPCK promoter (TgSREBP-1a), show a massively enlarged liver and atrophic peripheral white adipose tissue, as previously described [14]. Subsequent studies revealed that these phenotypes were influenced by age, sex, and especially dietary status. To control for these factors, we used male mice, aged 21 to 24 weeks, when these phenotypes were fully developed. Additionally, we maintained the mice on a chow diet, as the high protein/low carbohydrate diets could potentially disturb glucose metabolism.

3.1. TgSREBP-1a exhibits peripheral insulin resistance

As shown in Fig. 1, fasting plasma glucose levels were slightly, but significantly, higher in TgSREBP-1a (9.58±0.339 mmol/l) than in wild-type littermates (WT) (6.66±0.256 mmol/l) (Fig. 1A), and fasting insulin levels were significantly higher (550±82.0 ng/l) in the SREBP-1a transgenic mice compared to that in the WT controls (244±28.2 ng/l) (Fig. 1B). The homeostasis model assessment on insulin resistance (HOMA-R) [21] was 3-fold higher in TgSREBP-1a than in WT (5.54±0.816 vs. 1.78±0.238, respectively, Fig. 1C), indicating the presence of peripheral insulin resistance. Plasma glucose concentrations after intravenous glucose loading were showed slightly, but significantly, elevated in TgSREBP-1a animals throughout the test (Fig. 2A). Insulin levels in TgSREBP-1a mice were rapidly elevated following intravenous glucose loading, to levels higher than that of WT, and were maintained at higher levels than that of WT throughout the test (Fig. 2B). Calculated plasma glucose clearance rates normalized to insulin levels were significantly lower in TgSREBP-1a than in WT (Table 1). Finally, Tg-SREBP-1a mice demonstrated a diminished sensitivity to the insulin action of plasma glucose lowering than WT controls, as assayed in intraperitoneal insulin tolerance tests. These data confirm the presence of peripheral insulin resistance in TgSREBP-1a animals (Fig. 3).

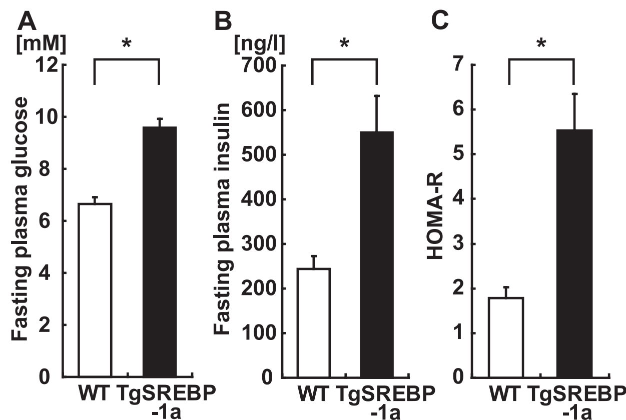

Fig. 1. Fasting plasma glucose and insulin levels and HOMA-R. Plasma glucose (A) and insulin (B) levels in animals after an overnight fast were determined. HOMA-R of each animal was calculated from fasting plasma glucose level and insulin level (HOMA-R=Fast PG [mM]×Fast INS [mU/l]/22.5). Open bars indicate WT and filled bars TgSREBP-1a. Data are presented as mean±S.E.; n=24 for TgSREBP-1a and 20 for WT on each measurement. *$P<0.01$ vs. WT.

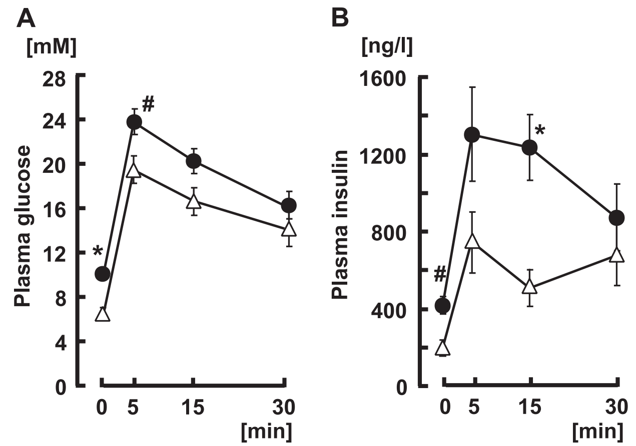

Fig. 2. Intravenous glucose tolerance tests in fasted SREBP-1a transgenic mice. Intravenous glucose tolerance tests were performed in both TgSREBP-1a and WT mice after an overnight fast. Glucose was injected intravenously via tail vein injection at a dose of 0.5g/kg body wt, and plasma glucose (A) and insulin (B) levels were measured. Filled circles (●) indicate plasma glucose and insulin levels of TgSREBP-1a, and open triangles (△) indicate those of WT. Data are shown as mean±S.E. (n=10 in TgSREBP-1a, 8 in WT). *$P<0.01$ vs. WT, #$P<0.05$ vs. WT.

3.2. Lipodystorophy caused peripheral insulin resistance, but did not increase plasma NEFA levels

Previous reports demonstrated that the SREBP-1a transgene, under the control of the PEPCK promoter, was expressed in adipose tissue as well as in the liver, leading to lipodystrophy [14]. Thus, we sought to determine whether the peripheral insulin resistance was caused by decreasing

Table 1
Plasma glucose clearance normalized to the AUC of insulin on ivGTTs

	Controls [Δμmol/ng insulin/min]	TgSREBP-1a [Δμmol/ng insulin/min]
Fasted	1.187±0.333 (6)	0.535±0.0769* (9)
Fed	0.326±0.0653 (5)	0.117±0.0397* (5)

Data are means±S.E. (n).
* $P<0.01$ compared with controls.

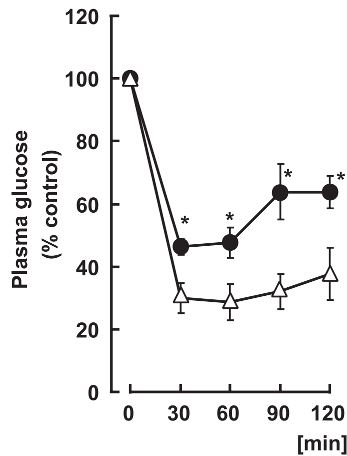

Fig. 3. Insulin tolerance in SREBP-1a transgenic mice. Intraperitoneal insulin tolerance tests were performed in both TgSREBP-1a and WT mice. After an overnight fast, a human insulin analogue was injected intraperitoneally at a dose of 2 units/kg body wt, and plasma glucose levels were measured. Percent ratios of plasma glucose levels vs. those of at 0 min are shown. Filled circles (●) indicate ratios of TgSREBP-1a and open triangles (△) are WT. Data are presented as mean percent±S.E. (n=10). *P<0.01 vs. WT.

fat mass [22]. The disappearance of adipose tissue is time-dependent and usually occurs by the time the mice are 12 weeks old. Therefore, the adipose tissue in the 21–24 week old transgenic mice used in the current study was atrophic. The lipodystrophy was likely the cause of differences in plasma leptin levels between TgSREBP-1a (1151±145.0 pg/ml) and WT (6201±918.0 pg/ml) (Fig. 4A) and could explain the peripheral insulin resistance [23–25]. This observation is similar to the phenotype of SREBP-1c transgenic mice, where the adipose-specific ap2 promoter was used to drive the transgene (aP2-TgSREBP-1c) [26], although the PEPCK-TgSREBP-1a mice display a much milder insulin resistance and no diabetes, whereas aP2-TgSREBP-1c showed severe insulin resistance and diabetes.

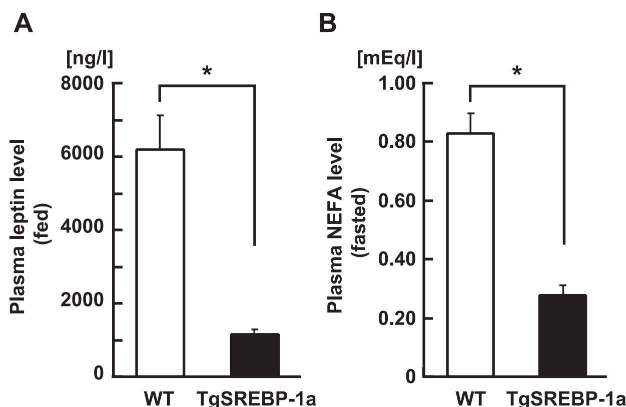

Fig. 4. Plasma leptin and NEFA levels. Leptin levels were measured on plasma obtained from fed animals, and NEFA levels were on fasted plasma samples. Open bars indicated WT and filled bars TgSREBP-1a. Data are presented as mean±S.E.; n=9 for TgSREBP-1a and 10 for WT on leptin measurements, and n=5 for NEFA measurements. *P<0.01 vs. WT.

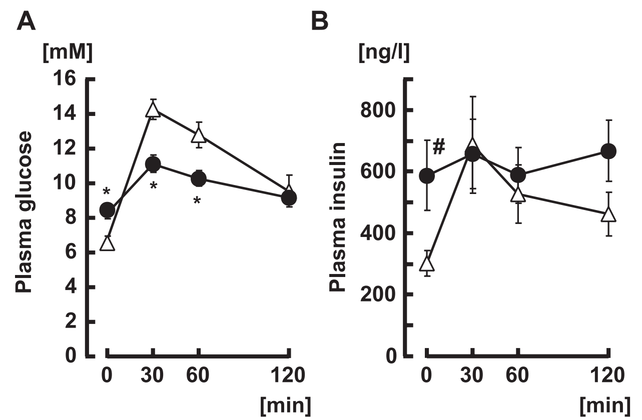

Fig. 5. Intraperitoneal glucose tolerance tests in fasted SREBP-1a transgenic mice. Intraperitoneal glucose tolerance tests were performed in SREBP-1a transgenic mice (TgSREBP-1a) and WT after an overnight fast. Glucose was injected intraperitoneally at a dose of 1 g/kg body wt, and plasma glucose (A) and insulin (B) levels were measured. Filled circles (●) indicate plasma glucose and insulin levels of TgSREBP-1a, and open triangles (△) indicate those of WT. Data are shown as mean±S.E. (A, n=25 each; B, n=20 in TgSREBP-1a, 17 in WT). *P<0.01 vs. WT, $^{\#}P$<0.05 vs. WT.

Elevated plasma NEFA levels are thought to exacerbate insulin resistance in peripheral tissues [27]; however, the PEPCK-TgSREBP-1a used in these studies showed lower plasma NEFA levels than those of the controls (Fig. 4B). Thus, we concluded that their insulin resistance was not due to the lipotoxicity of peripheral organs.

3.3. Livers of TgSREBP-1a mice have a large capacity for glucose tolerance

Fig. 5 shows the results of intraperitoneal glucose tolerance tests. After peritoneal injection of glucose, the elevation of plasma glucose in TgSREBP-1a was less marked than in WT, reversing the fasting difference at 30 and 60 min (TgSREBP-1a 10.9±0.566 mmol/l vs. WT 14.1±0.530 mmol/l, Fig. 5A), followed by achieving similar levels at 120 min. As shown by the 0 time point in Fig. 5B, fasting insulin levels are significantly higher (668±140 ng/l) in the SREBP-1a transgenic mice compared to that of the WT controls (340±51.2 ng/l). In contrast to the WT animals, which showed a typical pattern of a 30 min rise followed by falling insulin levels, the SREBP-1a transgenic animals did not show any significant changes in their insulin levels following glucose injection. The results demonstrate that TgSREBP-1a mice exhibit mild insulin resistance at fasting but have a larger capacity for glucose tolerance,

Table 2
The AUC of glucose normalized by the AUC of insulin on ipGTTs

	Controls [μmol glucose/ng insulin]	TgSREBP-1a [μmol glucose/ng insulin]
Fasted	29.9±3.68 (17)	24.3±3.70 (20)
Fed	6.20±0.980 (5)	1.70±0.312* (5)

Data are means±S.E. (n).
* P<0.01 compared with controls.

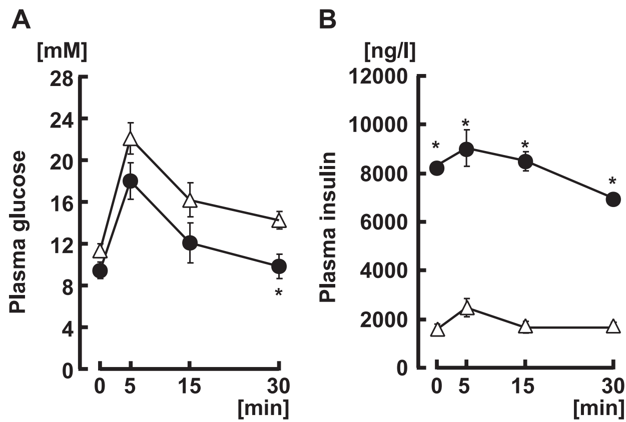

Fig. 6. Intravenous glucose tolerance tests in fed SREBP-1a transgenic mice. Intravenous glucose tolerance tests in TgSREBP-1a and WT were performed in ad libitum fed animals. After intravenous glucose injection via the tail vein at a dose of 0.5 g/kg body wt, plasma glucose (A) and insulin (B) levels were measured. Filled circles (●) indicate plasma glucose and insulin levels of TgSREBP-1a, and open triangles (△) indicate those of WT. Data are presented as mean±S.E. (A and B, *n*=5 respectively). **P*<0.01 vs. WT, #*P*<0.05 vs. WT.

presumably due to the larger liver mass and increased glucose incorporation into lipids via activity SREBP-1a, resulting in a diminished elevation in plasma glucose and a reduced insulin response. The calculated ratio of glucose area under the curve (AUC) over insulin AUC was similar between TgSREBP-1a and WT mice (Table 2).

As previously reported, IP injected glucose primarily reaches the liver through the portal vein or directly via the liver surface [28–30]. Previous investigation in conscious dogs revealed that glucose uptake by the liver is efficient after intraportal glucose administration, but very low after peripheral intravenous glucose infusion [31]. A striking contrast in the GTT results in TgSREBP-1a mice depending upon the route of glucose loading (Figs. 2 and 5) is consistent with this report. It is conceivable that the enlarged livers in TgSREBP-1a mice could extract larger amounts of glucose, resulting in diminished insulin secretion from β-cells. The hepatic uptake of [1-^{14}C] 2-deoxy-glucose (2-DG) after intravenous injection into fasted mice was examined, and it was found that there was no significant difference in the radioactivity per weight of liver between the transgenic and wild-type mice (data not shown). Liver weight in SREBP-1a transgenic mice is two- to threefold higher than in the wild type, suggesting that total glucose uptake by the liver should be increased in TgSREBP-1a to a similar extent. The enlarged TgSREBP-1a livers also exhibited a high capacity for glucose loading in the fed state (Figs. 6 and 7). This hypothesis explains the absence of full-blown diabetes in this insulin-resistant animal model developing both lipodystrophy and fatty liver.

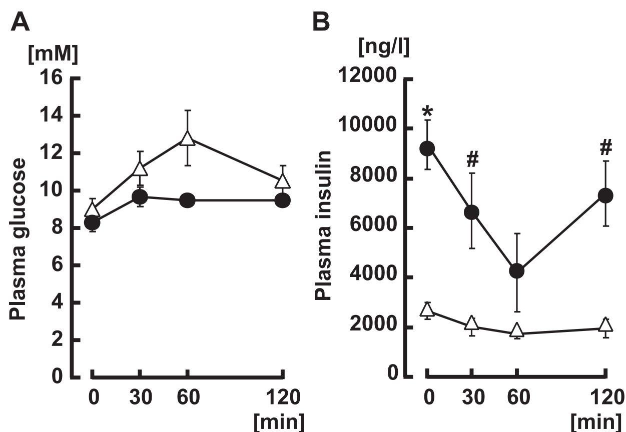

Fig. 7. Intraperitoneal glucose tolerance tests in fed SREBP-1a transgenic mice. During ad libitum feeding, intraperitoneal glucose tolerance tests in TgSREBP-1a and WT were performed. Glucose was injected intraperitoneally at a dose of 1g/kg body wt, and plasma glucose (A) and insulin (B) levels were measured. Filled circles (●) indicate plasma glucose and insulin levels of TgSREBP-1a, and open triangles (△) are for WT. Data are presented as mean±S.E. (*n*=5 for each). **P*<0.01 vs. WT, #*P*<0.05 vs. WT.

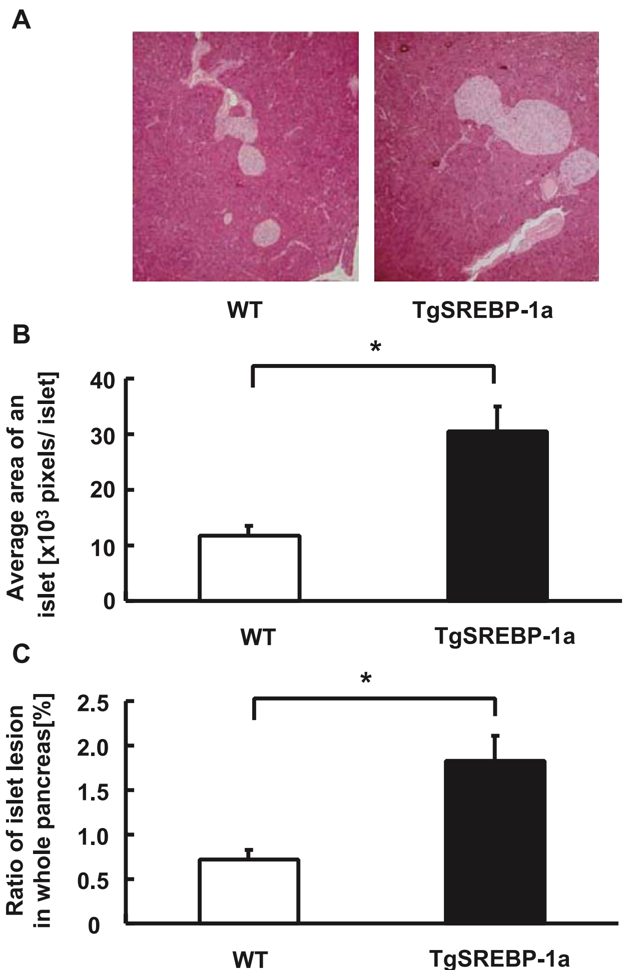

Fig. 8. Histological evaluations of pancreatic islets. Representative light microscopic photographs of pancreatic islets (hematoxylin–eosin stained) of WT and TgSREBP-1a (A), the comparison of average area of an islet counted by the pixels of digital image files (B), and the ratio of islet lesion in whole pancreas (C) in both WT and TgSREBP-1a are shown. Enlarged islets were observed in TgSREBP-1a (A). Open bar indicated WT and filled bar as TgSREBP-1a. Data are mean±S.E. from 51 islets from TgSREBP-1a and 52 islets from WT. **P*<0.01 vs. WT.

3.4. Hepatic SREBP-1a overproduction causes postprandial hyperinsulinemia

The results of ivGTT in animals fed ad libitum are shown in Fig. 6. Both groups showed a similar pattern of changes in plasma glucose, with minimally lower levels in TgSREBP-1a mice (Fig. 6A). The fourfold elevated insulin levels in fed TgSREBP-1a compared with those in WT were sustained throughout the test (Fig. 6B). The starting insulin level of TgSREBP-1a mice fed ad libitum were markedly higher than that of WT mice (8310±114 ng/l vs. 1568±227 ng/l), a much more prominent difference than was noted in fasted animals. Although the slight elevation in insulin levels was already significant at fasting, it was severely exacerbated in a fed state. Decreased glucose clearance rates in TgSREBP-1a after a glucose load were also more prominent in the fed condition than during fasting (Table 1), although plasma insulin levels in fed mice responded very little to plasma glucose changes in both groups. When ipGTT were performed in a fed state, WT mice showed a peak level of plasma glucose at 60 min, which was delayed and less prominent than the ipGTT on fasted WT mice. The plasma glucose in TgSREBP-1a remained constant and was slightly lower than in WT (Fig. 7A). Upon intraperitoneal glucose administration, insulin levels were considerably reduced in TgSREBP-1a (Fig. 7B), and WT insulin levels also slightly decreased. After 120 min, the insulin level in TgSREBP-1a mice recovered somewhat. The calculated glucose AUC over insulin AUC was markedly decreased in TgSREBP-1a as compared to that of WT, another indication of insulin resistance (Table 2). This unexpected amelioration of hyperinsulinemia after intraperitoneal glucose injection in TgSREBP-1a was also observed in *ob*/*ob* mice, but its mechanism is currently unknown [32]. This could be related to the hepatic clearance of plasma insulin. These postprandial data suggest that the overexpression of SREBP-1a promotes a more marked insulin resistance in the fed state, provoking insulin secretion.

3.5. Pancreatic islets were enlarged in TgSREBP-1a mice

Pancreatic islets were also examined in TgSREBP-1a mice. The mean size of the islets was significantly larger in TgSREBP-1a than in WT, as estimated by average pixel numbers per islet ($30.5 \pm 4.59 \times 10^3$ pixels vs. $11.7 \pm 1.88 \times 10^3$ pixels, Fig. 8). The density of the islet cells, as estimated by islet pixels/whole pancreas pixels, was also significantly higher in TgSREBP-1a (3.69±0.556%) than in WT (1.42±0.228%). The threefold increased mass of beta cells suggests the presence of continuous potent stimuli for insulin secretion in the mice overexpressing SREBP-1a. Because non-fasting plasma glucose was consistently lower than WT, their plasma glucose level could not be the main cause of this islet enlargement. It is possible that incretins could be involved in this hyperinsulinemia. The low plasma leptin levels of TgSREBP-1a could be partially involved, as leptin deficient *ob*/*ob* mice also show a similar progression of postprandial hyperinsulinemia, although *ob*/*ob* mice are diabetic [32]. It is more likely that some additional postprandial factor caused by hepatic SREBP-1a activation was superimposed on the basal peripheral insulin resistance in TgSREBP-1a mice, leading to severe hyperinsulinemia in a fed state. Recently, we found that SREBP overproduction can lead to hepatic insulin resistance through the suppression of IRS-2 [20], which potentially explains this.

The current data suggest that hepatic SREBP-1a activation could impact plasma insulin levels. Further investigations are needed to clarify the precise mechanisms. It could be related to the well-known clinical association of fatty liver and hyperinsulinemia in excess energy. In conclusion, the current studies suggest that the activation of SREBP-1a in the liver leads to compensatory hyperinsulinemia, which implicates new aspects to the role of hepatic SREBPs in insulin/glucose metabolism.

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