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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.

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).