Heterozygous knockout of the IRS-1 gene in mice enhances obesity-linked insulin resistance: a possible model for the development of type 2 diabetes

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Abstract

Insulin receptor substrate 1 (IRS-1) gene polymorphisms have been identified in type 2 diabetic patients; however, it is unclear how such polymorphisms contribute to the development of diabetes. Here we introduced obesity in heterozygous IRS-1 knockout (IRS-1+/−) mice by gold-thioglucose (GTG) injection and studied the impact of reduced IRS-1 expression on obesity-linked insulin resistance. GTG injection resulted in \( \sim 30\% \) weight gain in IRS-1+/− and wild type (WT) mice, compared with saline-injected controls. There was no difference in insulin sensitivity between lean IRS-1+/− and lean WT. Elevated fasting insulin levels but no change in fasting glucose were noted in obese IRS-1+/− compared with the respective lean controls. Importantly, fasting insulin in obese IRS-1+/− was 1.5-fold higher \((P<0.05)\) than in obese WT, and an insulin tolerance test showed a profound insulin resistance in obese IRS-1+/− compared with obese WT. The islets of obese IRS-1+/− were 1.4-fold larger than those of obese WT. The expression of insulin receptor and IRS-1 and IRS-2 was decreased in obese IRS-1+/−, which could in part explain the profound insulin resistance in these mice. Our results suggest that IRS-1 is the suspected gene for type 2 diabetes and its polymorphisms could worsen insulin resistance in the presence of other additional factors, such as obesity. Journal of Endocrinology (2002) 174, 309–319

Introduction

Type 2 diabetes mellitus is characterized by insulin resistance in peripheral tissues and failure of insulin secretion from pancreatic β-cells (O’Rahilly et al. 1995). Part of these abnormalities seem to be genetically defined. Several candidate genes for insulin resistance have been proposed, which include the genes encoding the molecules of the insulin signaling pathway (O’Rahilly et al. 1995), glycolysis (Ristow et al. 1999) and glycogen synthesis (Ludwig et al. 1996). In addition, some of the genes involved in obesity, including peroxisome proliferator-activated receptor-γ (Ristow et al. 1998) and uncoupling protein-1 (Heilbronn et al. 2000), may also contribute to insulin sensitivity.

Insulin receptor substrate-1 (IRS-1) is one of the major substrates of insulin receptor tyrosine kinase (Araki et al. 1993). After insulin stimulation, IRS-1 is immediately tyrosine phosphorylated by activated receptor tyrosine kinase, and some phosphorylated tyrosine residues of IRS-1 serve as docking sites for several src homology 2 (SH2)-containing proteins, including phosphatidylinositol 3-kinase (PI 3-kinase), SH2-containing protein tyrosine phosphatase-2 and growth factor-bound protein-2 (Myers et al. 1994, Argetsinger et al. 1995). Binding of these SH2 proteins to IRS-1 results in the activation of two major signaling pathways, the mitogen activated protein kinase pathway and the PI 3-kinase pathway, both of which mediate various biological effects of insulin.

The role of IRS-1 in insulin signaling in vivo has been confirmed by generating IRS-1 knockout mice by targeted disruption of the IRS-1 gene (Araki et al. 1994, Tamemoto et al. 1994). The IRS-1 homozygous knockout (IRS-1−/−) mice show impaired intrauterine growth, resistance to insulin and insulin-like growth factor-I (IGF-I), and glucose intolerance upon i.p. glucose loading. On the other hand, in spite of the low expression of IRS-1 protein, IRS-1 heterozygous knockout (IRS-1+/−) mice show no significant abnormalities in insulin sensitivity and glucose homeostasis.

Previous studies from our laboratories and those of other investigators described several polymorphisms of the
IRS-1 gene in patients with type 2 diabetes (Almind et al. 1993, Ura et al. 1996). Glycine to arginine substitution at codon 971 of IRS-1 gene (G971R) is the most frequent polymorphism in any population examined. The prevalence of G971R polymorphism is significantly higher in patients with type 2 diabetes than in normal subjects, and subjects who carry IRS-1 polymorphism tend to be insulin-resistant compared with those with no such mutation (Ura et al. 1996). Functional analysis of mutant IRS-1 confirmed that G971R mutant showed impaired insulin-induced PI 3-kinase activation and mitogenesis in the hematopoietic 32 D cell line (Almind et al. 1996, Yoshimura et al. 1997). These in vitro data strongly suggest that IRS-1 abnormalities could contribute to the development of insulin resistance in human. However, it is still not clear whether or how IRS-1 abnormalities could lead to insulin resistance in humans since most IRS-1 polymorphisms exist in a heterozygous manner in type 2 diabetes, whereas the heterozygous IRS-1 knockout mice show neither impairment of glucose homeostasis nor insulin sensitivity.

It is well known that environmental factors such as obesity and high fat diet are associated with increased prevalence of type 2 diabetes. In fact, the number of type 2 diabetes patients in Asian countries including Japan has markedly increased during the last few decades due to changes in life style (King & Rewers 1993). Furthermore, Clausen et al. (1995) reported that obese subjects who carry IRS-1 gene polymorphism are more resistant to insulin than those who have no mutation in the IRS-1 gene, whereas non-obese carriers of the IRS-1 gene polymorphism have normal insulin sensitivity. These findings suggest that the impact of IRS-1 gene polymorphism becomes overt in the presence of obesity. In order to examine the interaction between obesity and IRS-1 gene polymorphism, we induced obesity in IRS-1+/− mice by injection of gold-thioglucose (GTG). Using this model, we studied the impact of reduced IRS-1 expression on obesity-linked insulin resistance in vivo, and investigated the mechanisms that underlie insulin resistance in obese IRS-1+/− mice.

Materials and Methods

Establishment of obese IRS-1+/− mice

Animal care and experiments were carried out in accordance with institutional animal care regulations. IRS-1 heterozygous knockout mice (IRS-1+/−) were generated by mating female IRS-1−/− mice with male IRS-1−/− mice (Araki et al. 1994). The genotypes of mice were examined by PCR analysis after extraction of genomic DNA from the tail. Only male mice were used in this study. Obesity was induced by a single i.p. injection of 0.5 mg/g body weight of GTG (Sigma Chemical, St Louis, MO, USA) at 6 weeks of age. Mice were kept on a 12 h light:12 h darkness cycle, with the light cycle between 0700 and 1900 h. The mice were allowed free access to food and water.

Assessment of plasma parameters, i.p. glucose tolerance test and i.p. insulin tolerance test

Blood samples were obtained from snipped tail ends for determination of insulin and glucose levels. Blood glucose concentration was measured by the enzymatic assay (Arkray, Kyoto, Japan). Insulin concentration was measured by ELISA (Morinaga, Yokohama, Japan). An i.p. glucose tolerance test was performed after overnight fasting by administering 2.0 g glucose/kg body weight via the peritoneal cavity in conscious mice at 40 weeks of age. The area under the curve (AUC) was calculated as the integrated blood glucose level during the tolerance test. An i.p. insulin tolerance test was performed at 35 and 45 weeks of age by administering 1.0 U insulin/kg body weight. For a more precise assessment of insulin sensitivity, we calculated the area over the curve (AOC), which represented the integrated reduced level of blood glucose during the insulin tolerance test.

Morphological analysis of the pancreas

The pancreas was obtained from mice at 50 weeks of age, fixed in paraffin, and cut into 10 sections (5 µm thick) separated by 200 µm. Sections were immunostained with anti-insulin, anti-glucagon or anti-somatostatin antibodies (Nichirei, Tokyo, Japan). Biotinylated goat anti-rabbit IgG coupled to peroxidase and alkaline phosphatase, and streptavidin-biotin complex were used as secondary antibodies. 3’3’-Diaminobenzidine or Fast Red Substrate Kit (Nichirei) was used as substrate respectively. Sections were then counterstained with hematoxylin–eosin (HE) and visualized using an IX50 microscope (Olympus, Tokyo, Japan). At least 20 islets in each section were randomly selected and analyzed. The area of each islet was measured quantitatively with an image analysis software (NIH, Bethesda, MD, USA).

Immunoprecipitation and Western blotting

Mice were anesthetized with 100 mg/kg sodium pentobarbital injected i.p. Following the loss of pedal and corneal reflexes, saline or 5.0 U regular insulin were injected into the portal vein. The liver, gastrocnemius, and quadriceps muscles were dissected out 1 and 3 min after insulin injection, and immediately frozen in liquid nitrogen. The frozen tissue was homogenized in ice-cold lysis buffer (1% Triton X-100, 50 mM Heps, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 5 mM sodium vanadate, aprotinin (10 µg/ml), leupeptin (5 µg/ml) and polymethylsulfonyl fluoride (34 µg/ml)) using a Polytron PTA 20 S generator.
operated at maximum speed for 30 s. The insoluble material was removed by centrifugation at 125 000×g, in a 70 Ti rotor (Beckman Instruments, Inc., Fullerton, CA, USA) for 60 min, and equal amounts of the supernatant were subjected to immunoprecipitation for 120 min using the indicated antibodies. Subsequently, protein A-Sepharose was added and followed by incubation for another 60 min. The samples were processed for SDS-PAGE and Western blotting as described previously (Folli et al. 1993).

**PI 3-kinase assay**

Immune–complexed PI 3-kinase activity was measured by *in vitro* phosphorylation of phosphatidylinositol bisphosphates (PI) as described previously (Folli et al. 1993). Immune complexes were incubated in 45 μl reaction buffer containing 10 μM ATP, 30 μCi [γ-32P]ATP and 10 μg PI at room temperature under constant shaking. After 10 min, the reaction was stopped by the addition of 250 μl 1 M HCl and 80 μl CHCl3/methanol (2:1). The samples were centrifuged, and the lower organic phase was removed and applied to a silica gel TLC plate (Merck, Darmstadt, Germany) coated with 1% potassium oxalate. TLC plates were developed in CHCl3/CH3OH/H2O/NH4OH (60:47:11:3:2), dried, and visualized by autoradiography. The 32P-labeled PI was quantified using NIH image analysis software.

**Statistical analysis**

All data are expressed as means ± S.D. Differences between groups were examined for statistical significance using the non-parametric Mann–Whitney U-test. A *P* value less than 0.05 denoted the presence of a statistically significant difference.

**Results**

**Development of obesity following GTG administration**

Body weight of both IRS-1+/− and wild type (WT) mice increased by approximately 30% at 9 weeks after GTG administration, compared with the relative saline-injected control groups (IRS-1+/− group: 40.2 ± 2.6 g vs control: 33.0 ± 4.0 g, *P*<0.001, and WT group: 40.2 ± 3.4 g, vs control: 31.0 ± 4.7 g, *P*<0.001) (Fig. 1). There were no significant differences in body weight between obese WT and obese IRS-1+/− mice, and between lean WT and lean IRS-1+/− mice. The increase in body weight (∼30%) of the GTG-injected groups was maintained until 45 weeks of age.

**Fasting blood glucose and fasting serum insulin concentrations in WT and IRS-1+/− mice**

Fasting blood glucose and serum insulin concentrations were measured at 15 weeks of age and every 15 weeks thereafter. There was no significant difference in fasting blood glucose levels among four groups at 15 weeks of age (Fig. 2A). Blood glucose levels did not change significantly until 45 weeks of age in all groups (data not shown). There was no significant change in fasting serum insulin concentrations between lean WT and lean IRS-1+/− mice at 15 weeks (Fig. 2B). In contrast, the mean fasting serum insulin concentrations in obese WT mice were about three times higher than those of lean WT controls (1.62 ± 0.42 vs 0.56 ± 0.30 ng/ml, *P*<0.01). The mean insulin concentration of obese IRS-1+/− mice (2.56 ± 0.78 ng/ml) was also significantly higher than that of lean IRS-1+/− mice (0.65 ± 0.57 ng/ml, *P*<0.01). Importantly, fasting insulin concentrations in obese IRS-1+/− mice were significantly higher (*P*<0.05) than in obese WT mice.

**Insulin tolerance and glucose tolerance tests**

To assess glucose intolerance in IRS-1+/− mice, glucose (2.0 g/kg body weight) was injected i.p. at 40 weeks of age. Obese IRS-1+/− and obese WT mice showed a significant elevation in blood glucose concentrations after glucose loading compared with the respective lean controls (*P*<0.01) (Fig. 3). Furthermore, blood glucose levels in obese IRS-1+/− mice at 60 min after glucose challenge were higher than in obese WT mice. However, the AUC of obese WT mice (47 408 ± 3492) was not significantly different from that of obese IRS-1+/− mice (45 982 ± 2579).

![Figure 1 Growth curves for WT and insulin receptor substrate 1 (IRS-1)−/− mice following administration of gold-thioglucose (GTG). GTG or saline was injected into the peritoneal cavity of mice at 6 weeks of age. Body weight of GTG-injected WT (■) and IRS-1−/− (●) mice, and saline-injected WT (□) and IRS-1−/− (○) mice was measured until 45 weeks of age. Data are means ± S.D. of ten mice in each group. *P*<0.001 vs lean mice group.](Image 292x488 to 510x671)
We also assessed insulin sensitivity at 35 and 45 weeks of age by an i.p. insulin tolerance test. At 35 weeks of age, both obese groups showed a significant reduction in glucose-lowering effect of insulin compared with their lean controls (data not shown). Glucose levels after insulin challenge tended to be higher in obese IRS-1+/− mice than in obese WT mice, albeit statistically insignificant (data not shown). An i.p. insulin tolerance test at 45 weeks of age showed that both obese WT and obese IRS-1+/− mice exhibited a significant reduction in the glucose-lowering effect of insulin compared with their lean controls at all times after insulin challenge (Fig. 4). Importantly, the glucose-lowering effect of insulin in obese IRS-1+/− mice was markedly diminished
compared with obese WT mice. For a more precise assessment of the insulin-lowering effect, we calculated the AOC. The AOC of lean WT mice (1692 ± 201) was not significantly different from that of lean IRS-1+/− mice (1476 ± 201). However, the AOC of obese IRS-1+/− mice (315 ± 319) was significantly lower than that of obese WT mice (692 ± 333).

Morphological analysis of the pancreas

Islets in the pancreas section of obese WT and obese IRS-1+/− mice were significantly larger than those in their lean mice controls (Figs 5 and 6A). Interestingly, the mean islet size of obese IRS-1+/− mice was 36% larger than that of obese WT mice (P<0.05), whereas there was no difference in islet size between lean WT and lean IRS-1+/− mice (Figs 5 and 6A). Next we analyzed sections immunostained for insulin, glucagon and somatostatin. There was no obvious degranulation of β-cells in anti-insulin antibody stained sections from all groups. Furthermore, the relative number of β-cells in the islets was higher in obese IRS-1+/− mice than in obese WT mice (Figs 5 and 6B). In contrast, there was no significant difference in the number of non-β-cells between the two obese groups (Figs 5 and 6C).

Expression of insulin receptor and IRS-1 and IRS-2 in liver and skeletal muscles

To elucidate the molecular mechanisms of enhanced insulin resistance in obese IRS-1+/− mice, we examined the expression levels of insulin receptor and IRS-1 and IRS-2 proteins in the liver and muscles. Quantitative analysis showed no differences in the expression of insulin receptor between lean WT and lean IRS-1+/− mice, in both the liver and muscles (Fig. 7A and B). Furthermore, the expression of the insulin receptor in the liver and muscles of both obese groups was significantly lower than in their lean controls (by 28% in WT and by 32% in IRS-1+/− mice tended to be lower than that in obese WT, albeit statistically insignificant (P=0.07).

Discussion

In the present study, we induced hypothalamic obesity in IRS-1+/− mice and examined the impact of decreased IRS-1 expression on the obesity-linked insulin resistance in vivo. After i.p. administration, GTG is mainly transported to cells of the ventromedial hypothalamus and causes necrotic lesions in that area. In rodents, the GTG-induced hypothalamic lesion causes insulin resistance and obesity, which is mainly due to the associated hyperphagia (Le Marchand et al. 1994). Administration of GTG in IRS-1+/− mice resulted in increased food consumption and subsequent increase...
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in body weight, which was somewhat similar to that seen in GTG-injected WT mice. Our results also showed no significant difference in abdominal fat weight between obese IRS-1+/−/p1 and obese WT mice (data not shown). These findings suggest that heterozygous disruption of the IRS-1 gene does not have a major impact on hypothalamic lesion-induced hyperphagia and increased fat mass. Not only obese IRS-1+/−/p1 but also obese WT mice showed impaired response to blood glucose upon insulin challenge, demonstrating that insulin resistance is accompanied by hypothalamic obesity in these mice. Interestingly, although IRS-1+/− and WT mice acquire a similar increase of body weight, obese IRS-1+/−/p1 mice showed more profound insulin resistance than obese WT mice during the insulin tolerance test.

Despite the profound insulin resistance in obese IRS-1+/−/p1 mice, there was no difference in fasting blood glucose levels between obese IRS-1+/−/p1 and obese WT mice. This may be due to compensatory changes manifested by increased insulin secretion by increased numbers of pancreatic β-cells in obese IRS-1+/−/p1 mice. It is also of note that there was no significant difference in fasting blood glucose, fasting insulin levels, and islet size between lean IRS-1+/− and lean WT mice. These results indicate that the impact of heterozygous knockout of the IRS-1 gene on glucose homeostasis and insulin sensitivity becomes overt only after induction of obesity. Indeed, the additive effect of heterozygous disruption of the IRS-1 gene on insulin resistance and pathogenesis of diabetes is supported by the results that double-heterozygous knockout of the IRS-1 and insulin receptor gene in the mice is more diabetogenic than single hetero-knockout of each gene (Kido et al. 2000). In the present study, we observed upregulation of IRS-2 protein in both the liver and muscles in lean IRS-1+/−/p1 mice. This could in part explain the normal glucose tolerance and insulin sensitivity in lean IRS-1+/−/p1 mice.

Abnormalities of early steps of insulin signaling pathway are known to cause insulin resistance in vivo and in vitro. In animal models of insulin resistance as well as in diabetic subjects, decreased binding of insulin to the insulin receptor, decreased receptor kinase activity, and decreased expression levels of insulin receptor and IRS-1 and IRS-2 have been demonstrated in the liver and skeletal muscles (Kahn et al. 1973, Soli et al. 1975, Folli et al. 1993). To determine the mechanism that underlies the profound

**Figure 5** Morphological analysis of the pancreas obtained from 50-week-old mice. Hematoxylin-eosin (HE) stained (A–D) sections of the pancreas obtained from representative lean WT (A), lean IRS-1+/−/p1 (B), obese WT (C) and obese IRS-1+/−/p1 (D) mice. The samples were fixed, sectioned and stained with HE. Photographs show the typical islets of each group (magnification × 40). Immunohistochemical analysis of islets of obese WT and obese IRS-1+/−/p1 mice (E–H). Pancreases were obtained from 50-week-old mice, and sections were immunostained with anti-insulin (E, F) or anti-glucagon and anti-somatostatin cocktail antibodies (G, H). Photographs show the typical islets of each group (magnification × 100).

**Figure 6** Islet size and number of β-cells and non-β-cells in the pancreatic islets. Islet size (A), and number of β-cells (B) and non-β-cells (C) per islet were quantified as described in Materials and Methods. Data are means ± s.d. of five mice in each group. *P<0.05, **P<0.01 vs lean WT, ***P<0.001 vs lean IRS-1+/−/p1, ‡P<0.05.
Insulin resistance observed in obese IRS-1\(^{+/−}\) mice, we examined the expression of insulin receptor and IRS-1 and IRS-2 in the liver and striated muscles.

As shown in other obese animal models (Folli et al. 1993, Saad et al. 1993, Kerouz et al. 1997), the expression levels of insulin receptor and IRS-1 and IRS-2 in the liver and muscles of our obese mice groups were lower than in the respective lean controls. We found that, in addition to the decrease in IRS-1 expression, insulin receptor in the liver in obese IRS-1\(^{+/−}\) mice was significantly lower than in obese WT. It is also of note that IRS-2 expression in the liver and muscles was upregulated in lean IRS-1\(^{+/−}\) mice, and this upregulation was blunted in obese IRS-1\(^{+/−}\) mice. Based on the results of knockout mice of either the IRS-1 or IRS-2 gene, several investigators proposed that IRS-1 is an important substrate for the metabolic actions of insulin in the muscles while IRS-2 is necessary for insulin action in the liver (Araki et al. 1994, Tanemoto et al. 1994, Withers et al. 1998). The importance of the insulin receptor in the muscles and liver has also been confirmed by muscle- and liver-specific disruption of the insulin receptor in mice (Brüning et al. 1998, Wojtaszewski et al. 1999, Michael et al. 2000). Therefore, the decrease in insulin receptor and IRS-1 in both tissues and blunted upregulation of IRS-2 protein could explain the profound insulin resistance in obese IRS-1\(^{+/−}\) mice.

We also studied the PI 3-kinase activity, a key signal involved in insulin-induced glucose uptake in peripheral tissues and regulation of glyconeogenesis in the liver (Agati et al. 1998). Our results showed that PI 3-kinase activity in the liver and muscles was significantly reduced in obese mice. This reduction could be explained by the low expression of insulin receptor and IRS-1 and IRS-2 in both tissues. We also found a significant reduction in

Figure 7 Expression levels of insulin receptor and IRS-1 and IRS-2 in the liver and muscles. Equal amounts of protein obtained from the liver (left panels) and muscles (right panels) were separated by SDS-PAGE in duplicate and immunoblotted with anti-insulin receptor (IR) (A, B), anti-IRS-1 (C, D) or anti-IRS-2 (E, F) antibody. Representative results of Western blot analysis are shown above each bar plot, and quantitative analysis of the signals by NIH image analysis software is shown below the blots. The expression levels are relative to those of lean WT and expressed as means ± S.D. of five independent experiments (total ten animals). *P<0.05, **P<0.01, ***P<0.001 vs lean WT, †P<0.05, ††P<0.01, †††P<0.001 vs lean IRS-1\(^{+/−}\), ‡P<0.05, ‡‡P<0.01 vs lean WT.
Insulin-stimulated PI 3-kinase activity in the muscles of obese IRS-1+/− mice compared with obese WT mice. This could be, at least in part, due to reduced expression of IRS-1. However, an important question remains unanswered: what are the mechanisms that regulate the expression of these molecules? It is known that chronic insulin treatment downregulates the expression and/or phosphorylation of insulin signaling molecules, including insulin receptor and IRS-1 and IRS-2 (Friedman et al. 1997, Kerouz et al. 1997). In our model, the expression levels of insulin receptor and IRS-1 were low while serum insulin levels were high in obese IRS-1+/− and obese WT. At present, it is not clear how insulin receptor is downregulated under a chronic hyperinsulinemic state. On the other hand, previous studies proposed that downregulation of IRS-1 protein in the presence of hyperinsulinemia is due to the degradation of IRS-1 protein (Araki et al. 1995). It is also reported that IRS-2 expression is negatively regulated by insulin by suppressing gene transcription and enhancing protein degradation (Rui et al. 2001, Zhang et al. 2001). Interestingly, IRS-2 protein, which is upregulated to supernormal levels in lean IRS-1+/− mice, was reduced in obese IRS-1+/− mice, to levels as low as those seen in obese WT mice. It is possible that IRS-2 is upregulated in lean IRS-1+/− by reduction of the insulin signal through IRS-1, and chronic hyperinsulinemia could cause a downregulation of IRS-2 protein in both obese mice. It is also reported that factors produced by adipocytes, such as adipocytokines and free fatty acids, can modulate the
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Insulin signaling pathway (Hotamisligil et al. 1994, Dresner et al. 1999, Griffin et al. 1999). Further studies are therefore necessary to determine the precise mechanism(s) involved in the regulation of these proteins in our mouse models.

Although insulin resistance in obese IRS-1+/− mice was more severe than in obese WT mice, there was no significant difference in fasting glucose levels between these two groups of mice. This is probably due to the compensation by increased insulin secretion from hypotrophied pancreatic islets. The latter were larger in both groups of obese mice compared with their lean controls. Furthermore, the mean islet size of obese IRS-1+/− mice was significantly larger than that of obese WT mice, and this enlargement was, at least in part, due to an increased number of pancreatic β-cells. It is known that vagal hyperactivity induced by ventromedial hypothalamic lesions stimulates proliferation of pancreatic β-cells through a cholinergic receptor mechanism (Kiba et al. 1996). On the other hand, as shown in Fig. 6, the increase in islet mass (which was larger than that of the β-cell number per islet) suggested possible β-cell hypertrophy in obese IRS-1+/− mice. We examined the size of β-cells in some islets by microscopy, and found that obese IRS-1+/− mice tended to have larger β-cells compared with obese WT mice, albeit statistically insignificant (data not shown). Thus, we could not exclude a possibility of β-cell hypertrophy in obese IRS-1+/− mice. Considered together, the enlargement of each islet might be the result of both an increased number of β-cells and β-cell hypertrophy. It is known that other obese models such as db/db mice (Tomita et al. 1992) and lean insulin-resistant models such as IRS-1−/− mice (Brüning et al. 1997) also show an increase in islet mass. In our study, the size of islets paralleled the degree of insulin resistance in each mouse group. The regulation of pancreatic β-cell growth is not fully understood at present. It has been reported that the IGF-I signal mediated by IRS-2 is important for pancreatic β-cell growth (Schuppin et al. 1998), and recent studies proposed the involvement of Akt, a serine/threonine kinase activated by insulin or IGF-I stimulation, in β cell growth (Tuttle et al. 2001). Further studies are necessary to investigate the insulin signaling pathway including Akt activity in β-cells in our model.

In conclusion, we have demonstrated in the present study that GTG administration induced obesity and insulin resistance in both IRS-1+/− and WT mice. Importantly, obese IRS-1+/− mice were more insulin-resistant and had larger islets than obese WT mice, although insulin sensitivity of lean IRS-1+/− mice was similar to that of lean WT mice. Our results suggest that IRS-1 gene polymorphisms might be associated with worsening of insulin sensitivity in humans in the presence of additional factor(s), such as obesity. These findings widen the scope of our understanding of the mechanism involved in the development of type 2 diabetes mellitus in obese subjects.

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