A novel approach to assess insulin sensitivity reveals no increased insulin sensitivity in mice with a dominant-negative mutant hepatocyte nuclear factor-1α

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Ahren, B., and G. Pacini. A novel approach to assess insulin sensitivity reveals no increased insulin sensitivity in mice with a dominant-negative mutant hepatocyte nuclear factor-1α. Am J Physiol Regul Integr Comp Physiol 291: R131–R137, 2006. First published February 9, 2006; doi:10.1152/ajpregu.00519.2005.—In phenotype experiments in mice, determination of dynamic insulin sensitivity often uses the insulin tolerance test. However, the interpretation of this test is complicated by the counterregulation occurring at low glucose. To overcome this problem, we determined the dynamic insulin sensitivity after inhibition of endogenous insulin secretion by diazoxide (25 mg/kg) in association with intravenous administration of glucose plus insulin (the DSGIT technique). Estimation of insulin sensitivity index (SII) by this technique showed good correlation to SII from a regular intravenous glucose tolerance test (r = 0.87; P < 0.001; n = 15). With DSGIT, we evaluated dynamic insulin sensitivity in mice with a rat insulin promoter (β-cell-targeted) dominant-negative mutation of hepatic nuclear factor (HNF)-1α [RIP-DN HNF-1α (Tg) mice]. When insulin was administered exogenously at the same dose in Tg and wild-type (WT) mice, plasma insulin levels were higher in WT, indicating an increased insulin clearance in Tg mice. When the diazoxide test was used, different doses of insulin were therefore administered (0.1 and 0.15 U/kg in WT and 0.2 and 0.25 U/kg in Tg) to achieve similar insulin levels in the groups. Minimal model analysis showed that SII was the same in the two groups (0.78 ± 0.21 x 10^-4 min·pmol⁻¹·1⁻¹ in WT vs. 0.60 ± 0.11 in Tg; P = 0.45) as was the glucose elimination rate (P = 0.27). We conclude that J) the DSGIT technique determines the in vivo dynamic insulin action in mice, 2) insulin clearance is increased in Tg mice, and J) chronic islet dysfunction in RIP-DN HNF-1α mice is not compensated with increased insulin sensitivity.

glucose effectiveness; glucose tolerance; minimal modeling; diazoxide; insulin action

IN PHENOTYPE STUDIES IN MICE, dynamic insulin sensitivity is often evaluated by the insulin tolerance test (ITT), in which insulin is administered exogenously and the fall in glucose is recorded (8, 11). A problem with the ITT is, however, that it allows glucose to fall below baseline, which initiates a counterregulatory response, thus complicating the interpretation. To circumvent this, we have developed a method, which by adapting the minimal model of glucose disappearance allows the estimation of insulin sensitivity index (SII) during an intravenous glucose tolerance test (IVGTT) (1, 6, 21). In this technique, glucose is not reduced below baseline, which therefore prevents counterregulatory responses and thus avoids complication of the interpretations. The technique requires, however, a sufficient insulin response to the intravenous administration of glucose. For example, in a previous study, we examined insulin sensitivity in mice with severe reduction in insulin secretion (26). The mice have a monogenic severe β-cell dysfunction caused by a rat insulin promoter (β-cell-targeted) dominant-negative mutation of hepatic nuclear factor (HNF)-1α (RIP-DN HNF-1α) (9, 26). These mice are experimental tools for the study of the most common form of maturity-onset diabetes in the young (MODY), MODY3, which comprises 50–60% of all MODYs and is caused by a heterozygous mutation in the gene encoding the homeodomain-containing transcription factor HNF-1α (30). Patients with this type of diabetes have a progressive inhibition of insulin secretion with development of diabetes complications, if not properly treated (12). The RIP-DN HNF-1α mice exhibit severe bluntness of glucose-stimulated insulin secretion and low serum levels of insulin in association with hyperglycemia and glucose intolerance (9, 26). However, we found that in these mice it was not possible to quantify SII from a regular IVGTT because the suppression of insulin secretion was so severe that hardly any suprabasal elevation of plasma insulin followed the glucose challenge, making it impossible to use the model. In the present study, we have therefore developed a new method, the diazoxide-supplemented glucose-insulin test (DSGIT), for establishment of dynamic insulin sensitivity in mice and applied this technique for exploring insulin sensitivity in the RIP-DN HNF-1α mice. The DSGIT is based on the potent action of diazoxide to suppress insulin secretion, which has been reported both in vivo (21) and in vitro (14). This action is due to the ability of diazoxide to open the ATP-regulated K⁺ channels in the cells, which prevents Ca²⁺ from entering the β-cells (10, 13). Diazoxide is given before glucose, and this inhibits glucose from stimulating insulin secretion. Then, exogenous insulin is administered together with glucose to allow the needed increase in circulating insulin for the minimal model analysis. SII is then determined as the estimate of dynamic insulin sensitivity. As a comparison, we also determined static insulin sensitivity in RIP-DN HNF-1α mice by the euglycemic hyperinsulinenic clamp technique. The aim of this study was therefore the development of DSGIT and the evaluation of insulin sensitivity in the MODY3 mice model with severe primary β-cell dysfunction, such as the RIP-DN HNF-1α. We performed the study in female mice to minimize the confounding factor of severe diabetes because
there is a clear gender difference in the diabetes phenotype in RIP-DN-HNF-1α mice in that male mice have a more severe form of diabetes (9).

METHODS

Animals. The RIP-DN HNF-1α (Tg) mice and their wild-type (WT) controls were kind gifts of Dr. Claes Wollheim, University of Geneva (Geneva, Switzerland). As described previously, the DN-HNF-1α cDNA was inserted into a plasmid under the control of the RIP for construction of a RIP-DN HNF-1α transgene (9, 29). The transgenic mice were generated by pronuclear microinjection of the construct in B6/CBAJ-F1 × B6/CBAJ-F1 zygotes. Tg and WT mice were transported from the animal facility of the University Medical Center, Geneva, to the In Vivo Department, Biomedical Center, Lund University (Lund, Sweden) after embryo transfer was performed at Taconic (Ry, Denmark). The animals were cross-bred for >16 generations to C57BL/6J mice. Tg animals were identified by PCR on genomic DNA extracted from tail biopsies by the primer 5'-CTGCTAAACATGTTCATTGCT-3' and 5'-TGAATTTGAGCGACCTCTACTC-3'. The animals were kept in a 12:12-h light-dark schedule and constant infusion of 30 mU kg⁻¹ h⁻¹ of insulin. Controls were kind gifts of Dr. Claes Wollheim, University of Geneva.

Construction of a RIP-DN HNF-1α transgene. Tg mice exhibit a more severe form of diabetes than their female counterparts (9). Mice were anesthetized with an intraperitoneal injection of diazoxide (400 mg/kg; Sigma Chemical, St. Louis, MO) or saline was given as a subcutaneous injection. Thirty minutes later, diazoxide (25 mg/kg; Sigma Chemical, St. Louis, MO) or saline was given as a subcutaneous injection. Then, 10 min later, a 75-μL blood sample was taken from the retrobulbar, intraorbital, and capillary plexus in heparinized tubes, and d-glucose (British Drug Houses, Poole, UK; 1 g/kg) was given intravenously alone or together with human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark; 0.1–0.4 U/kg). In the series of experiments with IVGTT, the experiments were performed similarly, except that diazoxide and insulin were not injected. The volume load was 10 μL/g body wt. At specific time points after injection, 75-μL blood samples were collected, again from the retrobulbar, intraorbital, and capillary plexus in heparinized tubes. Blood was placed in heparinized tubes and immediately centrifuged, whereupon plasma was separated and stored at −20°C until analysis. For evaluation of insulin sensitivity in a euglycemic, hyperinsulinemic clamp test, the test was undertaken according to previous work (4, 20), as previously described (21). Mice were anesthetized as above. The right jugular vein and the left carotid artery were catheterized. The venous catheter was used for infusion of glucose and insulin, and the arterial catheter was used for sampling. Thirty minutes after introduction of the catheters, synthetic human insulin (Actrapid) was infused at a rate of 60 mU·kg⁻¹·min⁻¹ for 1 min followed by a continuous and constant infusion of 30 mU·kg⁻¹·min⁻¹. The volume load was 4 μL during minute 1 followed by 2 μL/min thereafter. Blood glucose levels were determined at 5-min intervals for 90 min by the glucose dehydrogenase technique with the use of a Hemocue (Hemocue, Angelholm, Sweden). A variable rate of glucose (40 g/dl solution) was infused to maintain blood glucose levels at the target of 6.5 mmol/L. A blood sample was taken at 90 min for the determination of plasma insulin.

Assays. Insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, with 125I-labeled human insulin as tracer and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/L, and the coefficient of variation is <3% within assays and <5% between assays. Plasma glucose concentrations were determined with the glucose oxidase technique.

Calculations and statistics. From data obtained with DSGIT, the suprabasal area under the 120-min insulin curve (AUCinsulin) was calculated with the trapezoidal rule; glucose tolerance was quantified by the glucose elimination constant (KGluc), calculated as the slope of the logarithmic transformation of circulating glucose between 5 and 20 min after the glucose bolus. The S1 and glucose effectiveness (S2) were estimated with the minimal model analysis (21). After we demonstrated that diazoxide inhibits endogenous insulin production, insulin clearance was evaluated as injected insulin dose divided by the AUCinsulin. During the euglycemic, hyperinsulinemic clamp test, insulin sensitivity was determined by the glucose infusion performed between minute 60 and 90 of the clamp. Data and results are reported as means ± SE. Statistical comparisons of data were performed with Student’s unpaired t-tests with Bonferroni correction for multiple comparisons. Pearson’s product moment correlation coefficients were obtained to estimate linear correlation between S1 from DSGIT vs. S1 from IVGTT.

RESULTS

Baseline body weight, glucose, and insulin. Body weight was not significantly different between the two groups, being 25.8 ± 0.3 g in WT (n = 89) and 26.5 ± 0.3 g in Tg (n = 83; P = 0.153). Nonfasting plasma glucose levels were higher in Tg (12.7 ± 0.4 mmol/l) than in WT mice (9.0 ± 0.2 mmol/l; P < 0.001), whereas insulin levels were lower in Tg (242 ± 13 pmol/l) than in WT mice (309 ± 14 pmol/l; P < 0.001).

Effects of diazoxide in IVGTT. The effect of diazoxide or saline, administered subcutaneously 10 min before the intravenous administration of glucose, is shown in Fig. 1. In WT mice, diazoxide markedly reduced the glucose-stimulated insulin secretion, which was followed by severe reduction in glucose tolerance, as evident by the higher glucose levels. In Tg mice, intravenous glucose did not yield any insulin response with a marked impairment of glucose tolerance. Diazoxide reduced even more insulin levels, worsening glucose tolerance, as evident by higher glucose levels. This shows that administration of diazoxide 10 min before glucose allows similar patterns of endogenous insulin in the two mice breeds.

Diazoxide-augmented IVGTT. To estimate S1 from IVGTT data using the minimal model, exogenous insulin was administered in mice subjected to diazoxide pretreatment. The same dose of 0.25 U/kg of insulin was initially given to WT and Tg mice. However, this resulted in significantly higher insulin levels in WT than in Tg mice (AUCinsulin, Table 1). KGluc (Table 1) was markedly higher in WT than in Tg mice (Fig. 2), and S2 did not differ between the two groups. Also, S3 was not significantly different between the groups (Table 1), suggesting that, despite the lower insulin in Tg, no compensatory increase in insulin sensitivity occurred in these mice. However, the unmatched insulin levels weaken the strength of this conclusion.

Effects of different doses of insulin. To circumvent the interpretative problem of different insulin levels in the two
groups during DSGIT, insulin was administered at various doses: 0.1 (n = 6), 0.15 (n = 6), or 0.25 (n = 18) U/kg in WT and 0.1 (n = 6), 0.2 (n = 6), 0.25 (n = 11), or 0.4 (n = 6) U/kg in Tg to achieve similar insulin levels in the two groups. It was evident throughout this dose range that in WT, higher insulin levels were achieved for the same dose of administered insulin (Fig. 3). This indicates that insulin clearance is higher in Tg mice (0.07 ml/min) vs. 0.4 ml/min in WT mice (n = 30); P < 0.001.

For a correct interpretation of estimated parameters between the two groups, model analysis was undertaken at similar insulin levels. WT mice underwent DSGIT with insulin doses of 0.1 or 0.15 U/kg (n = 12), whereas similar insulin levels were achieved by Tg at the doses of 0.2 or 0.25 U/kg (n = 17). Figure 4, which reports the pooled experiments with the two doses, shows that glucose levels also did not differ between the two groups. \( K_G \), \( S_I \), and \( S_G \) (Table 2) were, in fact, not different, indicating that Tg mice have dynamic insulin sensitivity and glucose tolerance not different from those of their WT counterparts.

Comparing DSGIT with IVGTT in normal and insulin-resistant mice. In one series of experiments, female C57BL/6J mice were given a high-fat diet (58% fat from lard) for 8 wk, which is an established method for induction of insulin resistance (21, 26). In these mice and control mice given normal diet, a regular IVGTT with estimation of \( S_I \) was undertaken followed by a DSGIT with \( S_I \) estimation 2 wk later. Figure 5 shows the glucose and insulin responses; it is seen that high-fat diet was associated with glucose intolerance and that insulin levels were augmented compared with mice fed normal diet (Table 3). \( S_I \) was reduced in mice fed the high-fat diet compared with mice fed the normal diet. From IVGTT (P = 0.002) and from DSGIT (P = 0.027) results, although \( S_I \) was numerically determined to be higher in IVGTT than in DSGIT (P = 0.015 in mice fed the normal diet and P = 0.046 in mice fed the high-fat diet), we see in Fig. 6 that \( S_G \) obtained from the two techniques correlated significantly (r = 0.83; P < 0.001). \( S_G \)

Table 1. Parameters derived from glucose and insulin analysis with minimal model of DSGIT when insulin was injected at the dose of 0.25 U/kg in WT and Tg mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT Mice (n = 18)</th>
<th>Tg Mice (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{\text{glucose}}) mmol/l × 75 min</td>
<td>44.2±1.9</td>
<td>30.3±1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(K_G) (minutes 5-20), %/min</td>
<td>4.6±0.4</td>
<td>2.7±0.5</td>
<td>0.007</td>
</tr>
<tr>
<td>(S_I) 10(^{-4}) min(^{-1}) mmol(^{-2}) F(^{-1})</td>
<td>0.82±0.11</td>
<td>0.75±0.15</td>
<td>0.697</td>
</tr>
<tr>
<td>(S_G) kg-1 min(^{-2})</td>
<td>0.042±0.005</td>
<td>0.056±0.013</td>
<td>0.363</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mice. DSGIT, diazoxide-supplemented glucose-insulin test; AUC\(_{\text{glucose}}\), area under the curve for insulin; \(K_G\), glucose elimination constant; \(S_I\), insulin sensitivity index; \(S_G\), glucose effectiveness; Tg, transgenic (from rat insulin promoter \(\beta\)-cell-targeted dominant-negative mutation of hepatic nuclear factor); WT, wild type. P indicates probability levels of random difference between the groups.

Fig. 1. Plasma glucose and insulin levels (means ± SE) before and after an intravenous injection of glucose (1 g/kg) with a preceding subcutaneous injection of saline or diazoxide (25 mg/kg) 10 min before glucose in wild-type mice (WT mice; n = 19 and 30, respectively; A) and in mice with a rat insulin promoter (\(\beta\)-cell-targeted) dominant-negative mutation of hepatic nuclear factor (HNF)-1α [RIP-DN HNF-1α (Tg mice); n = 25 and 29, respectively; B]. Probability levels of random difference between respective groups: *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 2. Plasma glucose and insulin levels (means ± SE) before and after an intravenous injection of glucose (1 g/kg) and insulin (0.25 U/kg) with a preceding subcutaneous injection of diazoxide (25 mg/kg) 10 min before glucose (1g/kg) in WT mice (n = 18) and in Tg mice (n = 11). Probability levels of random difference between groups: *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 3. Relation between administered insulin dose in the diazoxide-supplemented glucose-insulin test (DSGIT) and the area under the curve for insulin (AUC\(_{\text{insulin}}\)) in WT mice (n = 30) and in Tg mice (n = 29).
was numerically not significantly different between the two tests ($P = 0.076$ in mice fed the normal diet and $P = 0.061$ in mice fed the high-fat diet).

**Euglycemic, hyperinsulinemic clamp.** During the clamp test, insulin was infused at 30 mU·kg$^{-1}$·min$^{-1}$ in WT and Tg mice. This raised plasma levels of insulin at 90 min to $11.0 \pm 1.2$ nmol/l in WT mice ($n = 7$) and to $9.4 \pm 0.7$ nmol/l in Tg mice ($n = 9$; not significant). Glucose was infused to maintain blood glucose levels at $\approx 6.5$ mol/l. Figure 7 shows the blood glucose levels during the tests. The mean glucose levels during minutes 60–90 were $6.2 \pm 0.1$ nmol/l in WT mice vs. $6.3 \pm 0.2$ nmol/l in Tg mice (not significant). During minutes 60–90, glucose infusion was $18.3 \pm 2.1$ mg·kg$^{-1}$·min$^{-1}$ in WT mice and $17.5 \pm 1.2$ mg·kg$^{-1}$·min$^{-1}$ in Tg mice (not significant). The glucose infusion divided by the insulin level during the clamp was $11.1 \pm 3.0$ nmol (glucose/kg)/(pmol insulin/l) in WT mice and $11.0 \pm 1.3$ nmol (glucose/kg)/(pmol insulin/l) in Tg mice (not significant).

**DISCUSSION**

In this report, we present a novel method to assess dynamic insulin sensitivity in mice that uses diazoxide to modulate insulin concentration at the level of peripheral tissues. With this novel approach, we have shown that insulin sensitivity is not increased in Tg mice to compensate their lifelong marked reduction of insulin secretion. This compromised upregulation of insulin sensitivity may therefore contribute to the diabetic conditions in these mice. In a previous study, our group (26) sought to establish insulin sensitivity in Tg mice by estimating $S_I$ during an IVGTT. It was, however, not possible to estimate $S_I$ because of the severe islet dysfunction in these mice, which resulted in a markedly reduced, almost abolished, insulin response to glucose; a robust increase in insulin during the IVGTT is required for estimation of $S_I$. In this study, we therefore developed a new method for studying insulin sensitivity under in vivo conditions in mice, in which an increase in endogenous insulin is not required: the DSGIT. The technique is based on the potent action of diazoxide to suppress insulin secretion (14, 21). The insulin action in this model is examined when insulin is administered together with glucose. This allows the estimation by minimal model analysis of the insulin $S_I$ and $S_G$, which quantifies the action of glucose per se to modulate its own disappearance. These parameters, along with $K_G$, provide an integrated picture of the main factors contributing to the glucose disappearance rate in mice after an intravenous glucose administration (1, 6, 21).

To determine insulin sensitivity in the Tg mice, DSGIT was necessary because these mice have such compromised $\beta$-cell function that there is a severe bluntness of the insulin response to glucose, making it impossible to estimate $S_I$ from conventional use of the minimal model technique (26). However, in mice with a normal $\beta$-cell function, DSGIT also offers advantages over commonly used methods for estimation of insulin sensitivity in vivo. A common technique that has been used in previous mouse studies is the ITT (8, 11). This technique is based on the glucose-reducing action of insulin and adopts the rate fall or nadir in glucose levels as end points. The use of the

**Table 2. Parameters derived from glucose and insulin analysis with minimal model of DSGIT when insulin was injected at the doses of 0.1 or 0.15 U/kg in WT and at 0.20 or 0.25 U/kg in Tg mice**

<table>
<thead>
<tr>
<th></th>
<th>WT Mice ($n = 12$)</th>
<th>Tg Mice ($n = 17$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{insul}$, nmol/l × 75 min</td>
<td>27.6±1.8</td>
<td>29.3±1.2</td>
<td>0.433</td>
</tr>
<tr>
<td>$K_G$ (minutes 5–20), %/min</td>
<td>3.2±0.5</td>
<td>2.9±0.5</td>
<td>0.269</td>
</tr>
<tr>
<td>$S_I$, $10^{-4}$ min$^{-1}$·pmol$^{-1}$·l$^{-1}$</td>
<td>0.78±0.21</td>
<td>0.60±0.11</td>
<td>0.445</td>
</tr>
<tr>
<td>$S_G$, min$^{-1}$</td>
<td>0.055±0.005</td>
<td>0.056±0.013</td>
<td>0.363</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ = no. of mice. Pooled data from the 2 doses of insulin in the respective groups are shown. $P$ indicates probability levels of random difference between the groups.
DSGIT, as developed here, has several advantages over the ITT. First, it shows the activity of insulin to suppress a raised glucose level, which is a more physiological condition than the action of insulin to reduce glucose below basal. Second, and most importantly, it shows the effect of insulin under conditions when hypoglycemia counterregulation is not activated. Third, the DSGIT allows a wider dose range of insulin to be examined because supraphysiologically raising the insulin levels in the ITT may be even lethal because of the consequent severe hypoglycemia. In contrast, the regular IVGTT, which has been frequently used in mouse studies for estimation of insulin sensitivity (1, 2, 21), relies on the insulin concentration that is achieved as a result of the glucose bolus; therefore, insulin action at other insulin concentrations cannot be estimated. Nevertheless, a good correlation between the two techniques exists, as estimated in mice given a normal and a high-fat diet, which is a model for insulin resistance (26). Furthermore, a well-established technique in mice is the euglycemic, hyperinsulinemic clamp technique (21). This technique is, however, more complicated and cannot be undertaken on a large-scale basis; it is also afflicted with the conceptual problem of determining insulin action under static conditions when glucose levels are constant, whereas DSGIT and IVGTT estimate insulin sensitivity under dynamic conditions. A similar test is the rapid insulin sensitivity test, which has been established in rats and has been shown to be reliable in estimating insulin sensitivity at euglycemic conditions (18). However, the rapid insulin sensitivity test has not yet been explored in mice.

Several limitations of the DSGIT technique may exist. One such limitation is that non-steady-state insulin and glucose levels occur after the rapid administration. This is important when interpreting the results because SI obtained under these conditions is an estimate of dynamic insulin sensitivity, which sometimes may be different from a steady-state condition as obtained during the euglycemic, hyperinsulinemic clamp technique. Another potential limitation of the technique is that diazoxide has hemodynamic effects that may result in hypoglycemia. Yet another potential limitation is that hyperglycemia, which is evident during the test, may itself have effects on glucose turnover independent from insulin action but affecting the estimation of SI. However, SI was not significantly different in DSGIT vs. regular IVGTT, suggesting that this effect is minimal. A potential limitation of the test would also be that diazoxide itself could affect insulin action. However, our group (21) previously showed that diazoxide does not affect insulin sensitivity during a euglycemic, hyperinsulinemic clamp test. Insulin sensitivity as determined by the IVGTT (SI) correlates to that obtained from the euglycemic clamp (21), and here we show that SI from IVGTT and DSGIT correlate (r = 0.83). Therefore, DSGIT seems reliable for the in vivo purpose of determining insulin sensitivity under dynamic conditions.

A primary goal of this study was to examine whether the lifelong and severe suppression of insulin secretion in Tg is associated with a compensatory increase in insulin sensitivity. It is known that there is an inverse relation between insulin sensitivity and insulin secretion, such that during insulin resistance there is a compensatory increase in insulin secretion (for review, see Ref. 2). The upregulation of insulin secretion has been demonstrated under a variety of conditions, such as in

![Fig. 6. Linear regression for insulin sensitivity index (SI) as determined by the IVGTT vs. SI as determined by the DSGIT in C57BL/6J mice given a normal diet (n = 7) or a high-fat diet for 8wk (n = 8).](image)

![Fig. 7. Blood glucose concentrations during the euglycemic, hyperinsulinemic clamp test in WT mice (n = 7) or in Tg mice (n = 9). Values are means ± SE.](image)
obesity (16) or after experimental induction of insulin resistance by dexamethasone (17). Conversely, it has been demonstrated that elite athletes having increased insulin sensitivity exhibit a compensatory reduction in insulin secretion (3). The inverse relation between insulin sensitivity and insulin secretion has also been demonstrated in experimental models in mice (1, 21). The inverse relation would imply that not only a primary alteration in insulin secretion is followed by the reciprocal change in insulin sensitivity but also that a primary change in insulin secretion is reciprocally adapted in insulin sensitivity, and it may be hypothesized that a compromised upregulation of insulin sensitivity in low insulin secretion may contribute to diabetes. However, whether a primary reduction in insulin secretion is adapted by increased insulin sensitivity is not known. To explore this in the Tg mice, we applied the DSGIT. We found that insulin levels were different in Tg than with WT mice, despite injecting the same dose of hormone. Because insulin was higher in WT than in Tg mice, it may be hypothesized that Tg mice exhibit an increased degradation of exogenously administered insulin. Insulin degradation is a complex process, which mainly takes place in the liver and the kidney, although all insulin-sensitive tissues remove insulin from the circulation for degradation (for a review, see Ref. 5). The mechanism by which Tg mice, which have low insulin levels, exhibit an increased clearance is therefore puzzling and not known at present. Evidence shows that insulin degradation is related to insulin action (7, 19, 24), and this may be explained by the hormone degradation by the hormone-sensitive tissues. However, studies have also shown that the great majority (80%) of total insulin is bound to liver receptors (15); therefore, internalization of insulin should affect $S_I$ only marginally because $S_I$ is mostly peripheral and determined by dynamic conditions.

The unexpected finding of altered insulin levels after administration of the same amount of insulin in the Tg compared with the WT mice suggests that it is important to administer different amounts of insulin in the two groups of mice to match insulin levels when estimating insulin action. Although it has been shown in humans that insulin levels do not matter for the correct estimation of $S_I$ (22), we believe that assessing $S_I$ at matching insulin levels would avoid a possible confounding factor in the interpretation of the minimal model results. This is not restricted to the use of DSGIT and would be important also in ITT. This requirement for the ITT is sometimes overlooked; therefore, studies reporting such data should be viewed cautiously. By simply calculating $K_C$ after exogenous administration of insulin may, in fact, lead to erroneous conclusions.

In the present study, we therefore administered insulin at several different dose levels to allow correct comparison between the groups, and we showed that metabolic parameters of glucose disappearance, especially $S_I$, are not impaired in Tg animals. Hence, Tg mice do not exhibit an increase of insulin sensitivity as a response to the impaired islet function. We also showed this with the euglycemic, hyperinsulinemic clamp test. This is a similar finding, as in a previous study in subjects with MODY-type diabetes, who were found to exhibit insulin sensitivity not different from healthy subjects, albeit higher than that shown in subjects with Type 2 diabetes (28). Similarly, in mice with heterozygous β-cell-specific genetic deletion of glucokinase, the severe suppression of insulin secretion is not compensated with increased insulin action; in contrast, a hepatic insulin resistance is seen during a hyperglycemic clamp (23), which, however, may be due to long-standing hyperglycemia. This suggests that insulin sensitivity is not increased to compensate for the low insulin secretion in mouse diabetes models with primary β-cell defect. The mechanism for the failure in Tg to augment insulin action and that of glucose per se is not clear. A possible explanation is that the long-standing β-cell dysfunction negatively perturbs glucose tolerance and insulin action, possibly because of the glucose homeostasis dysfunction. This will aggravate the diabetes condition; therefore, long-standing MODY may be associated with combined islet dysfunction and insulin resistance, although the β-cell seems to be primarily affected.

In conclusion, this study has shown 1) that DSGIT is a method for the in vivo determination of the dynamic insulin action in mice, 2) that insulin clearance is increased in our Tg mice, and 3) that the chronic islet dysfunction in our Tg mice is not compensated with increased insulin sensitivity, making this a good model for studying diabetes with β-cell dysfunction and compromised increase in insulin sensitivity.

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GRANTS

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