The Continuous Low Dose Insulin and Glucose Infusion Test: A Simplified and Accurate Method for the Evaluation of Insulin Sensitivity and Insulin Secretion in Population Studies


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ABSTRACT

In this study we investigated a simple nonlabor-intensive method to evaluate insulin sensitivity and β-cell function which is suitable for application in population studies. The method is a refinement of the modified Harano test and consists of a continuous low dose insulin (25 mU/kg.h) and glucose (4 mg/kg.min) infusion test (LDIGIT) lasting 150 min. Insulin sensitivity was evaluated as the MCR of glucose divided by the steady state serum insulin level achieved at the end of the test. Insulin secretion was expressed as the incremental area for C-peptide concentration during the first 15 min of the test. We compared the indices of insulin sensitivity and insulin secretion yielded by LDIGIT with those derived from the euglycemic clamp and the hyperglycemic clamp, respectively. Fifty-four subjects underwent a LDIGIT (33 with normal glucose tolerance and 21 with impaired glucose tolerance); of the 54, 19 were submitted to a euglycemic clamp, and 10 to a modified Harano test (insulin infusion, 50 mU/kg.h; glucose infusion, 6 mg/kg.min). LDIGIT overcame the drawbacks associated with the modified Harano test because it resulted in more stable final glucose levels and prevented the occurrence of hypoglycemic episodes. No significant differences were found between the insulin sensitivity index (ISI) of the LDIGIT and that of the euglycemic clamp for each group of subjects. Moreover, there was a strong correlation between the ISI determined by LDIGIT and the ISI determined by clamp (r = 0.90; P < 0.0001), and the best regression line was not different from the identity line, suggesting that the two indices are equivalent. The index of insulin secretion provided by LDIGIT correlated well with that of the hyperglycemic clamp (r = 0.82; P < 0.001) and was significantly higher in overweight subjects than in normal weight subjects. In conclusion, LDIGIT is a simple and accurate method to assess insulin sensitivity and secretion. It can be useful in population studies and in situations when more complex techniques are not feasible. (J Clin Endocrinol Metab 80: 34–40, 1995)

POPULATION studies for the measurement of insulin sensitivity and insulin secretion in subjects at increased risk of NIDDM, obesity, and hypertension are of primary importance to design preventive strategies. The euglycemic-hyperinsulinemic clamp and the hyperglycemic clamp are the gold standard for the assessment of insulin sensitivity and insulin secretion, respectively (1, 2). Recently, it has been shown that the hyperglycemic clamp may be used to assess both insulin sensitivity and secretion in the same individual in a single experiment (3). On the other hand, the hyperglycemic clamp is not feasible for investigating insulin sensitivity and insulin secretion in large cohorts of subjects, because it is costly and labor intensive. An alternative method to assess insulin sensitivity and insulin secretion is the frequently sampled iv glucose tolerance test analyzed by the minimal model (4, 5). The results obtained with this test have been validated (6, 7), and the recent development of a reduced sampling schedule makes it suitable for population studies (8). However, the indices derived from the minimal model are not immediately available from experimental data, because their evaluation requires the computer-assisted analysis of the glucose and insulin data sets. Thus, it would be desirable to have an alternative method capable to minimize both the experimental effort and the computational analysis. To this respect, a candidate method for the assessment of insulin sensitivity is that proposed by Harano et al. (9) and subsequently modified by Heine et al. (10). The modified Harano test, which builds upon the original insulin suppression test developed by Reaven et al. (11, 12), employs a continuous insulin and glucose infusion test and takes the plasma glucose level achieved at the end of the test as an index of insulin sensitivity. The modified Harano test is simple and requires few blood samples, and the interpretation of its results in terms of insulin sensitivity is straightforward. In addition, because during the modified Harano test, both insulin and C-peptide are released by the pancreas in response to the initial elevation of the plasma glucose concentration, this test could be feasible for the evaluation of β-cell function as well. Among its drawbacks, however, are the difficulty of achieving a steady state blood glucose level...
C-peptide determinations were taken at -10, -5, 0, 5, 10, 15, 30, 45, 60, 90, 120, 130, 140, 145, and 150 min. To evaluate possible changes in counterregulatory hormones during LDIGIT, samples for plasma glucose were taken before and after 60, 120, and 150 min of the tests in 17 normotolerant (13 normal weight and 4 overweight) and 16 intolerant (3 normal weight and 13 overweight) subjects.

In a subgroup of 8 subjects (4 NGT and 4 IGT), hepatic glucose production was also measured before and during LDIGIT. A primed (0.5 mg/kg) continuous infusion of (0.05 mg/kg/min) of [6,6-2H]glucose was started 2 h before and continued throughout the test. The [6,6-2H]glucose was also added to the unlabeled glucose infusion (700 mg in 250 mL 20% glucose solution) to maintain atomic percent enrichment constant at the basal level (17). The rate of appearance of unlabeled glucose was calculated using Steele's model (18) by calculating the stable isotope equivalent of specific activity, i.e., the tracer to tracee ratio (19). The tracer to tracee ratio was calculated from the atomic percent enrichment using the kinetic formalism developed in (19). A pool fraction of 0.65 and a total distribution volume of 260 mL/kg were used. Hepatic glucose production was derived by subtracting the known constant glucose infusion rate from the rate of appearance of unlabeled glucose.

Subjects and Methods

The protocol of the study was approved by the local ethics committee, and informed consent was obtained from all subjects. We studied 54 nondiabetic subjects, whose clinical details are shown in Table 1. All subjects underwent an OGTT (75 g) according to WHO criteria (16); 35 subjects showed normal glucose tolerance (NGT), and 21 showed impaired glucose tolerance (IGT). All subjects underwent a LDIGIT. Of these subjects, 19 were submitted to a euglycemic clamp, 18 to a hyperglycemic clamp, and 10 to a modified Harano test.

All tests were performed at 0900 h, with subjects fasted overnight. On the morning of each test, a 20-gauge cannula (Abbocath T, Abbocat, Ireland LTD, Sling, Ireland) was inserted retrograde in a dorsal vein of one hand, and the hand was maintained in a plexiglass box at 55°C for intermittent sampling of arterialized blood. Another 20-gauge plastic cannula, used for 20% glucose and insulin infusions, was placed in a large antecubital vein.

Modified Harano test and LDIGIT

Although the modified Harano test (10) consists of a continuous infusion of insulin (50 mU/kg·h) and glucose (6 mg/kg·min), the LDIGIT employs lower infusion rates of both insulin (25 mU/kg·h) and glucose (4 mg/kg·min). The new insulin and glucose infusion rates were designed to overcome the drawbacks of the modified Harano test, i.e., lack of stability of the final glucose level and hypoglycemic episodes in highly sensitive individuals. As the study by Heine et al. (10) has shown that endogenous insulin secretion does not change the insulin sensitivity levels in normal and IGT subjects, somatostatin was not used to inhibit insulin secretion in either test. During both tests, samples for blood glucose were taken at -10, -5, 0, 10, 15, 30, 40, 50, 60, 90, and 120 min and every 5 min until 150 min. Samples for serum insulin and serum C-peptide determinations were taken at -10, -5, 0, 5, 10, 15, 30, 45, 60, 90, 120, 130, 140, 145, and 150 min. To evaluate possible changes in counterregulatory hormones during LDIGIT, samples for plasma glucose, somatostatin was not used to inhibit insulin secretion in either test. During both tests, samples for blood glucose were taken at -10, -5, 0, 10, 15, 30, 40, 50, 60, 90, and 120 min and every 5 min until 150 min. Samples for serum insulin and serum C-peptide determinations were taken at -10, -5, 0, 5, 10, 15, 30, 45, 60, 90, 120, 130, 140, 145, and 150 min. To evaluate possible changes in counterregulatory hormones during LDIGIT, samples for plasma glucose, insulin, and C-peptide were taken at the same times as those for the LDIGIT.

Euglycemic clamp

The euglycemic clamp consisted of a 10-min glucose priming to raise blood glucose to 5.5 mmol/L above the basal level, followed by a variable glucose (20%) infusion to maintain blood glucose levels at 10 mmol/L for 150 min. Blood glucose concentrations were measured every 5 min for arterialized venous blood, and hyperglycemia was maintained within 10% of the target levels for an additional 140 min by means of a variable infusion of a 20% glucose solution. Samples to measure serum insulin and serum C-peptide levels were taken every 5 min from the first 15 min and every 15 min thereafter.

Hyperglycemic clamp

The hyperglycemic clamp consisted of a 10-min glucose priming to raise blood glucose to 5.5 mmol/L above the basal level, followed by a variable glucose (20%) infusion to maintain blood glucose levels at 10 mmol/L for 150 min. Blood glucose concentrations were measured every 5 min for arterialized venous blood, and hyperglycemia was maintained within 10% of the target levels for an additional 140 min by means of a variable infusion of a 20% glucose solution. Samples to measure serum insulin and serum C-peptide levels were taken every 5 min from the first 15 min and every 15 min thereafter.

Methods of evaluating insulin sensitivity

We compared insulin sensitivity indices (ISI) obtained during LDIGIT and the euglycemic clamp. Insulin sensitivity during the euglycemic clamp (ISIclamp) was obtained by dividing the average glucose infusion rate during the last 30 min of each clamp (M value) by the steady state plasma insulin concentration during the same interval (Iclamp). The tracer to tracee ratio was calculated from the atomic percent enrichment using the kinetic formalism developed in (19). Under the hypothesis that hepatic glucose production is totally suppressed at the end of the clamp, the M value coincides with the glucose disappearance rate, and the ratio M/Iclamp measures the plasma clearance rate of glucose. Thus, ISIclamp was expressed as glucose clearance per plasma insulin concentration (milliliters per kg/min per pmol/L): ISIclamp = M/Iclamp (Eq 1).

Insulin sensitivity during the LDIGIT (ISI LDIGIT) was obtained by dividing the constant glucose infusion rate (Gclamp) by the steady state insulin concentration during the last 30 min of each test (Iclamp) and

\[
\text{ISI}_{\text{LDIGIT}} = \frac{G_{\text{clamp}}}{I_{\text{clamp}}}
\]

TABLE 1. Clinical characteristics and fasting metabolic and hormonal levels of the subjects

<table>
<thead>
<tr>
<th>n (M/F)</th>
<th>Age (yr)</th>
<th>BMI (kg/m^2)</th>
<th>DG (mmol/L)</th>
<th>IRI (pmol/L)</th>
<th>C-Peptide (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight NGT</td>
<td>22 (11/11)</td>
<td>36 ± 2</td>
<td>22.2 ± 0.4</td>
<td>4.0 ± 0.1</td>
<td>36.6 ± 3.4</td>
</tr>
<tr>
<td>Normal weight IGT</td>
<td>4 (3/1)</td>
<td>28 ± 5</td>
<td>22.9 ± 0.6</td>
<td>5.2 ± 0.8</td>
<td>40.4 ± 4.3</td>
</tr>
<tr>
<td>Overweight NGT</td>
<td>11 (3/8)</td>
<td>49 ± 4</td>
<td>28.7 ± 1.0</td>
<td>4.7 ± 0.2</td>
<td>13.7 ± 10.2</td>
</tr>
<tr>
<td>Overweight IGT</td>
<td>17 (8/9)</td>
<td>48 ± 2</td>
<td>29.9 ± 0.6</td>
<td>4.9 ± 0.1</td>
<td>7.7 ± 8.8*</td>
</tr>
</tbody>
</table>

BMI, Body mass index; BG, blood glucose; IRI, serum insulin. Values are the mean ± se. Overweight: men, BMI > 27 kg/m^2, women, BMI > 25 kg/m^2.

* P < 0.01 vs. NGT normal weight; P < 0.05 vs. IGT normal weight.
normalizing this ratio to the steady state glucose concentration in the same period (Gss). Under the hypothesis that hepatic glucose production is totally suppressed at the end of the LDIGIT experiment, Ginf coincides with the glucose disappearance rate, and the ratio Ginf/Gss measures the plasma clearance rate of glucose. Thus, ISI_{LDIGIT} was expressed as glucose clearance per plasma insulin concentration (milliliters per kg/min-pmol/L): ISI_{LDIGIT} = Ginf/(Gss \times I) \quad \text{(Eq II)}.

The difference in the assessment of insulin sensitivity with LDIGIT and the euglycemic clamp is as follows. During the LDIGIT, glucose is infused at a constant rate whereas the glucose concentration varies until it reaches a steady state (Gss). By contrast, during the euglycemic clamp, the glucose concentration is maintained constant at the basal level, whereas the glucose infusion rate is varied until it reaches a steady state (M value).

**Methods of evaluating insulin secretion**

To determine whether LDIGIT would be feasible for examining β-cell function, we compared LDIGIT prediction for β-cell function with the analogous value derived from the hyperglycemic clamp. Insulin secretion was evaluated by taking the incremental area for the C-peptide concentration during the first 15 min of either LDIGIT or the hyperglycemic clamp.

**Assays**

Serum insulin (intraassay coefficient of variation (CV), 3.0%; interassay CV, 5.0%), serum C-peptide (intraassay CV, 3.0%; interassay CV, 3.0%), and plasma glucagon levels (intraassay CV, 3.1%; interassay CV, 3.0%) were measured by RIA, using commercial kits (Incstar-Sorin, Saluggia, Italy; Tecnogenetics, Cassina de Pecchi, Italy; and Byk-Gulden, Farmaceutici e Diagnostici SPA, Milan, Italy, respectively). Isotopic enrichment of [6,6-2H]glucose was measured using the gas chromatographic-mass spectrometric method previously reported (20).

**Statistical analysis**

All data are reported as the mean ± sd. The incremental areas (0–15 min) for C-peptide were calculated using the trapezoidal rule. Mean levels of each variable during the last 30 min of each test were considered for comparison. Comparisons among different groups were made by one-way analysis of variance as required, followed by Scheffe’s F test. Relationships were evaluated using simple Pearson correlation coefficients. P < 0.05 was considered statistically significant.

**Results**

Table 1 shows fasting levels of blood glucose, serum insulin, and C-peptide. Blood glucose levels were similar in all groups, whereas the differences in serum insulin and C-peptide levels between overweight and normal weight subjects were statistically significant. No differences were found between normal weight NGT and IGT subjects or between overweight NGT and IGT subjects.

**Modified Harano test**

During the last 30 min of the modified Harano test, the steady state blood glucose levels were 3.8 ± 0.3 mmol/L in normal weight NGT subjects and 7.5 ± 0.9 and 7.3 ± 1.4 mmol/L in overweight NGT and IGT subjects (P < 0.05 vs. normal weight NGT subjects). The CV of blood glucose levels was 4.2 ± 0.3%. The steady state serum insulin levels were 372.6 ± 16.8 pmol/L in normal weight NGT subjects and 441.6 ± 28.2 and 668.4 ± 43.8 pmol/L in overweight NGT and IGT subjects, respectively (P < 0.05, IGT vs. normal weight NGT subjects).

**LDIGIT**

During the LDIGIT, blood glucose levels increased in all groups during the first 45–60 min, declining thereafter in the NGT group and remaining higher in the IGT group (Fig. 1). Among the subjects of the NGT group, normal weight subjects showed lower steady state blood glucose levels than overweight subjects (4.3 ± 0.2 vs. 6.4 ± 0.4 mmol/L; P < 0.01). No differences were demonstrated between normal weight and overweight IGT subjects. (7.8 ± 0.3 vs. 7.4 ± 0.4 mmol/L; P = NS) The CV of blood glucose levels during LDIGIT was 2.6 ± 0.2%, significantly lower than that of the modified Harano test (P < 0.02 vs. modified Harano test). Serum insulin levels rose during the test, achieving a plateau at 174.9 ± 10.6 and 197.0 ± 27.2 pmol/L in normal weight NGT and IGT subjects, respectively (P = NS). The steady state serum insulin levels achieved in overweight NGT and IGT subjects were 354.1 ± 27.9 and 439.8 ± 42.0 pmol/L (P < 0.01), respectively. Serum C-peptide levels rose in all groups during the first 15–30 min, returning to basal values only in the normal weight NGT group and remaining higher in overweight NGT and in IGT subjects (Fig. 1).

Fasting plasma glucagon levels were similar in all groups (104.0 ± 9.5 and 128.4 ± 34.4 pg/mL in normal weight NGT and IGT subjects; 98.6 ± 26.3 and 131.0 ± 16.7 pg/mL in overweight NGT and IGT subjects; P = NS among groups) and did not change significantly during the test. During the last 30 min of the test, plasma glucagon levels were 97.4 ± 7.6 and 89.8 ± 17.9 pg/mL in normal weight NGT and IGT subjects and 92.4 ± 36 and 103.3 ± 11.0 in overweight NGT and IGT subjects, respectively (P = NS vs. basal values; P = NS among groups).

Fasting hepatic glucose production was similar in the NGT and IGT subjects (2.22 ± 0.10 and 2.21 ± 0.06 mg/kg-min, respectively). During the last 30 min of the test, hepatic glucose production was completely inhibited in both NGT and IGT subjects (103.8% and 96.0%, respectively).

**Euglycemic clamp**

During the euglycemic clamp, blood glucose levels were successfully clamped at the basal levels. The M value in the normal weight NGT subjects was significantly higher than that in the other groups of subjects (4.5 ± 0.3 vs. 2.3 ± 0.3 in overweight NGT and 2.6 ± 0.6 in overweight IGT; P < 0.05). The CV of blood glucose levels during the euglycemic clamp was similar to that obtained during LDIGIT (2.5 ± 0.5% vs. 2.6 ± 0.2%; P = NS) and significantly lower than that obtained during the modified Harano test (P < 0.05). The steady state serum insulin levels were 162.0 ± 12.0 pmol/L in normal weight NGT subjects and 350.4 ± 61.8 and 255.0 ± 11.4 pmol/L in overweight NGT and IGT subjects, respectively (P < 0.05, NGT overweight vs. NGT normal weight).

**Hyperglycemic clamp**

During the hyperglycemic clamp, blood glucose levels were maintained at the target levels (10 mmol/L), with a CV less than 3%. The M value was 9.8 ± 0.6 mg/kg-min in normal weight NGT subjects and 7.1 ± 1.5 and 5.5 ± 0.9 mg/kg-min in overweight NGT and IGT subjects.

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ASSESSMENT OF INSULIN SENSITIVITY AND SECRETION

NORMAL WEIGHT SUBJECTS

OVERWEIGHT SUBJECTS

Fig. 1. Blood glucose, serum insulin, and C-peptide levels during low dose insulin-glucose infusion test (LDIGIT) in normalweight (left) and overweight (right) subjects. NGT, normal glucose tolerance; IGT, impaired glucose tolerance. (* P < 0.01 NGT vs. IGT subjects)
respectively ($P < 0.05$, IGT overweight vs. NGT normal weight). The steady state serum insulin levels were $329.4 \pm 43.8$ pmol/L in normal weight NGT subjects and $391.8 \pm 88.2$ and $302.4 \pm 43.2$ pmol/L in overweight NGT and IGT subjects ($P = NS$), respectively.

Comparison of insulin sensitivity values measured by LDIGIT and euglycemic clamp

Table 2 shows the $\text{ISI}_{\text{LDIGIT}}$ results in normal weight and overweight subjects. In normal weight NGT subjects, the $\text{ISI}_{\text{LDIGIT}}$ was 2- to 3-fold higher than in the other groups ($P < 0.01$). The relationship between $\text{ISI}_{\text{LDIGIT}}$ and $\text{ISI}_{\text{clamp}}$ is shown in Fig. 2. We found a strong correlation between these two indices ($r = 0.90; P < 0.0001$). Of additional interest is the regression equation: $\text{ISI}_{\text{clamp}} = 1.97 + 1.08 \times \text{ISI}_{\text{LDIGIT}}$. The intercept (1.97) was not different from 0.00 ($P > 0.2$). Thus, for the group of subjects as a whole, the two measures of insulin sensitivity, $\text{ISI}_{\text{LDIGIT}}$ and $\text{ISI}_{\text{clamp}}$, are equivalent. In addition, there were no significant differences between $\text{ISI}_{\text{LDIGIT}}$ and $\text{ISI}_{\text{clamp}}$ for each group of subjects (Table 3). During both tests, the ISI in normal weight NGT subjects was significantly higher than that in the other groups ($P < 0.05$). There were no significant differences among the other groups.

Comparison of $\beta$-cell secretion values measured by LDIGIT and hyperglycemic clamp

The incremental areas for C-peptide calculated during first 15 min of both LDIGIT and hyperglycemic clamp were significantly correlated ($r = 0.82; P < 0.001$; Fig. 3). Table 2 shows the incremental areas for C-peptide during the first 15 min of the LDIGIT. The incremental areas for C-peptide were significantly higher in overweight NGT and IGT subjects than in normal weight NGT subjects despite a similar incremental area for glucose in the same period in all groups (data not shown).

Our results show that the euglycemic clamp and LDIGIT provide equivalent estimates of insulin sensitivity when the results yielded by the two protocols are expressed in terms of glucose clearance per plasma insulin concentration. We found a strong and highly significant correlation between $\text{ISI}_{\text{clamp}}$ and $\text{ISI}_{\text{LDIGIT}}$ ($r = 0.90, P < 0.0001$). In addition, the slope of the relationship was not different from unity, and the intercept was indistinguishable from zero, suggesting that the two indices are equivalent.

The calculation of both $\text{ISI}_{\text{clamp}}$ and $\text{ISI}_{\text{LDIGIT}}$ is based on the assumption that hepatic glucose release is completely suppressed during the last 30 min of each study. Under this condition, the exogenous glucose infusion rate coincides with the glucose disappearance rate, and $\text{ISI}_{\text{clamp}}$ and $\text{ISI}_{\text{LDIGIT}}$ correctly measure the plasma glucose clearance per unit serum insulin. Data from the literature indicate that, at the insulin levels achieved in our euglycemic clamp studies, hepatic glucose release is totally suppressed in NGT and IGT subjects (21, 22). To test whether the assumption of complete suppression of hepatic glucose release holds for LDIGIT as well, we measured hepatic glucose release during LDIGIT in a subset of four normal and four IGT subjects. We found that hepatic glucose release is indeed completely suppressed in NGT and IGT subjects.

**TABLE 2.** LDIGIT indices of insulin sensitivity and $\beta$-cell secretion

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>$\text{ISI}_{\text{LDIGIT}}$ ($\text{mL/kg} \cdot \text{min}/\text{pmol/L}) \times 10^3$</th>
<th>$\Delta \text{AUC} (0-15 \text{ min})$ ($\text{C-pep (nmol/L)} / \text{min}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight NGT</td>
<td>21</td>
<td>$31.8 \pm 2.0^{a,b}$</td>
<td>$3.14 \pm 0.29^{a}$</td>
</tr>
<tr>
<td>IGT</td>
<td>4</td>
<td>$15.7 \pm 2.5$</td>
<td>$3.15 \pm 0.79$</td>
</tr>
<tr>
<td>Overweight NGT</td>
<td>12</td>
<td>$11.8 \pm 1.5$</td>
<td>$5.86 \pm 0.73$</td>
</tr>
<tr>
<td>IGT</td>
<td>17</td>
<td>$8.9 \pm 1.2$</td>
<td>$4.29 \pm 0.63$</td>
</tr>
</tbody>
</table>

$\Delta \text{AUC}$, Change in the area under the curve (see Materials and Methods). Values are the mean ± SE.

$^{a}P < 0.01$ vs. NGT overweight.

$^{b}P < 0.01$ vs. IGT subjects.

**TABLE 3.** Comparison between ISI estimated from LDIGIT and the euglycemic clamp

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>$\text{ISI}_{\text{LDIGIT}}$ ($\text{mL/kg} \cdot \text{min}/\text{pmol/L}) \times 10^3$</th>
<th>$\text{ISI}_{\text{clamp}}$ ($\text{mL/kg} \cdot \text{min}/\text{pmol/L}) \times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight NGT</td>
<td>9</td>
<td>$32.1 \pm 3.4^{a}$</td>
<td>$37.3 \pm 3.9^{a}$</td>
</tr>
<tr>
<td>Overweight NGT</td>
<td>6</td>
<td>$9.5 \pm 2.1$</td>
<td>$11.2 \pm 3.9$</td>
</tr>
<tr>
<td>IGT</td>
<td>4</td>
<td>$12.0 \pm 2.6$</td>
<td>$14.5 \pm 4.1$</td>
</tr>
</tbody>
</table>

Values are the mean ± SE.

$^{a}P < 0.05$, normal weight vs. the other groups.
Our results show that LDIGIT is also suitable for the measurement of insulin secretion. There was a significant correlation between the LDIGIT and hyperglycemic clamp estimates of β-cell function (r = 0.82, P < 0.001). Insulin secretion indices obtained with LDIGIT were 30–40% lower than those found during the hyperglycemic clamp, probably due to the fact that the glucose infusion rate used with LDIGIT (4 mg/kg-min) was approximately 3-fold lower than that used with the hyperglycemic clamp (14 mg/kg-min). Our data indicate that LDIGIT is able to discriminate insulin secretion between normal weight and overweight subjects. In fact, the insulin secretion index yielded by LDIGIT was significantly lower in normal weight NGT than in overweight NGT and IGT subjects. These differences were not related to different incremental blood glucose levels during this period.

LDIGIT overcomes some of the difficulties encountered using the modified Harano test, as it results in a more stable final glucose level and prevents hypoglycemic episodes. In fact, during the LDIGIT, the coefficient of variation of the final glucose level was significantly lower than that during the modified Harano test and was comparable to that obtained during the euglycemic clamp. Moreover, whereas hypoglycemic episodes are occasionally observed in normal subjects during the final period of the modified Harano test (1, 14, 15), no hypoglycemic episodes were experienced with LDIGIT. Glucagon levels were not modified during the LDIGIT in all groups of subjects, confirming that counter-regulatory hormones were not stimulated by the blood glucose levels achieved during the final steady state period. Both of these improvements over the Harano test are due to a better design of the infusion rates of insulin and glucose. In particular, a relatively low insulin infusion rate was adopted, which was, however, still able to stimulate glucose utilization in insulin-sensitive tissues (23, 24). At these levels, the non-insulin-mediated glucose utilization becomes less important than the insulin-mediated glucose utilization. In a previous study we showed that the noninsulin-mediated glucose utilization is 70% of the total muscle glucose uptake at insulin levels of 42 pmol/L and decreases to 33% at insulin levels of 150–180 pmol/L (24). Hence, at the insulin levels experienced during the LDIGIT (200–400 pmol/L) insulin-mediated glucose utilization plays a predominant role in total glucose utilization.

The frequently sampled iv glucose tolerance test analyzed with the minimal model (4, 5) is an alternative approach to measure insulin sensitivity and insulin secretion in population studies because its metabolic indices have been validated, and a simplified protocol with a reduced number of samples has been recently developed (8). The minimal model method provides a portrait of glucose tolerance that is more detailed than that provided by LDIGIT, as not only insulin sensitivity, but also glucose effectiveness per se, can be determined. However, whereas the minimal model method entails the use of a rather sophisticated procedure to provide these metabolic indices, the LDIGIT evaluation of insulin sensitivity is straightforward. In fact, the minimal model parameters are not available directly from the measured data as in the LDIGIT, but they need to be disclosed by computer-assisted analysis of the insulin and glucose data sets. This analysis requires trained personnel to be performed adequately and becomes labor intensive, especially when the number of subjects is elevated as in population studies.

In conclusion, LDIGIT is a simple nonlabor-intensive method to assess insulin sensitivity and secretion. It involves low dose infusions of glucose and insulin at fixed rates for 150 min. LDIGIT yields an index of insulin sensitivity equivalent to that measured with the euglycemic clamp and an index of first phase insulin secretion highly correlated with the analogous parameter estimated during a hyperglycemic clamp. This method could be useful to characterize the
pathophysiology of glucose tolerance in population studies and in situations when more complex techniques are not feasible.

References