Comparison of Two Commonly Used Standard IVGTTs

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OBJECTIVE — To compare the magnitude and reproducibility of the FPIR measured during two different IVGTT protocols in nondiabetic subjects.

RESEARCH DESIGN AND METHODS — Nine control subjects each had two pairs of IVGTTs with either a 4-min infusion of 0.5 g/kg glucose or a 1-min infusion of 0.3 g/kg glucose. Blood glucose and serum insulin were measured before and 1, 2, 3, 5, and 10 min after completion of the glucose infusion. The FPIR was measured with either 1 + 3-, 2 + 3 + 5-, or 1 + 3 + 5-min serum insulins, areas under the insulin curve (0–5 or 0–10 min), or the ratio of serum insulin to blood glucose area.

RESULTS — The FPIR was higher in eight of nine subjects with the short-infusion test, but the within-subject variation of the two methods was identical. Reproducibility was not significantly improved with an integrated insulin area or insulin-to-glucose ratio measurement.

CONCLUSIONS — Reproducibility of the FPIR measured during IVGTT is not significantly affected by the duration of the glucose infusion. However, the magnitude of the difference in FPIR observed between the two protocols highlights the need for standardization of the methodology if the IVGTT is to be used in studies of the preclinical stage of IDDM.

The FPIR to glucose, measured during IVGTT, is being used increasingly in studies of the preclinical stage of IDDM. Srikanta et al. (1) demonstrated a linear decline in FPIR (sum of insulin at 1 and 3 min after glucose i.v.) before development of diabetes in ICA^+ relatives. They found that in adults FPIR may be <1st percentile for nondiabetic control subjects as long as 4 yr before the onset of clinical diabetes. Similar findings have been reported by Maclaren et al. (2). However, another study suggested that the FPIR has a high within-subject variation, which may limit its use in the prediction of IDDM and in monitoring intervention treatment (3). Another study documented that age and pubertal status also influence the interpretation of FPIR (4).

A major unresolved problem with IVGTT is a lack of uniform methodology, making comparison of results between centers difficult. In this study, we aimed to quantitate differences between the FPIR in two commonly used IVGTT protocols (1,5) and compare their within-subject precision. At a time when standardization of the antibody tests (ICA and insulin autoantibody) used in prediabetes studies has been achieved, the findings have relevance for guiding a choice of a standard protocol for IVGTT.

RESEARCH DESIGN AND METHODS

Nine healthy nondiabetic subjects (3 men, 6 women; mean age 31 yr [range 22–43 yr]) first had two IVGTTs with a long-infusion protocol. Within at least 3 mo, each subject had a second pair of IVGTTs with a short-infusion protocol. Each pair of tests was performed 1–2 wk apart.

IVGTTs

Tests commenced between 0800 and 0930 and were performed by the same research nurse. Subjects were asked to consume a normal diet for 3 days before each test, to avoid smoking, and to fast from 2200 the night before the test. A 19-gauge butterfly catheter, inserted into an antecubital vein, was used for both infusion of the glucose and blood sampling.

IVGTT protocols

Long-infusion protocol. Glucose (0.5 g/kg body wt) as a 25% solution was

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IVGTT, INTRAVENOUS GLUCOSE TOLERANCE TEST; FPIR, FIRST-PHASE INSULIN RESPONSE; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ICA, ISLET CELL ANTIBODY; CV, COEFFICIENT OF VARIATION.

infused intravenously under gravity over 3.5–4.5 min. Blood was collected immediately before and 1, 2, 3, 5, and 10 min after completion of the infusion.

Short-infusion protocol. Glucose (0.3 g/kg body wt) as a 25% solution was manually injected intravenously at a uniform rate over 1 min. Blood was collected immediately before and 1, 2, 3, 5, 7, and 9 min after completion of the injection. Time zero was defined as the termination of glucose infusion. Samples for blood glucose were collected into fluoride-heparin tubes and assayed the same day on a Beckman CX3 analyzer (Beckman, Brea, CA). Samples for serum insulin were placed on ice immediately, separated, and frozen at −20°C until assay. Insulin was assayed with a double-antibody radioimmunoassay (INCSTAR, Stillwater, MN). The interassay CVs were 4% at 2.8 mM and 2.1% at 18.6 mM for glucose and 10% at 11 mU/L, 7.3% at 31 mU/L, and 6.5% at 81 mU/L for insulin.

Calculation of FPIR

FPIR was measured as the sum of the serum insulins at 1 and 3 min; 2, 3, and 5 min; and 1, 3, and 5 min after completion of the infusion, and the area under the serum insulin curve from 0 to 5 and 0 to 10 min for the long infusion and from 0 to 9 min for the short infusion, and the ratio of serum insulin area to blood glucose area over 0–10 min for the long infusion and 0–9 min for the short infusion. For the short infusion, the area was calculated from the start of the infusion, whereas for the long infusion test, to avoid underestimation, the area was calculated from midway through the infusion (i.e., at −2 min).

Statistics

SD and CV were used as measures of between- and within-subject variations. Student's *t* tests were used to compare the mean blood glucose and serum insulin levels at different time points.

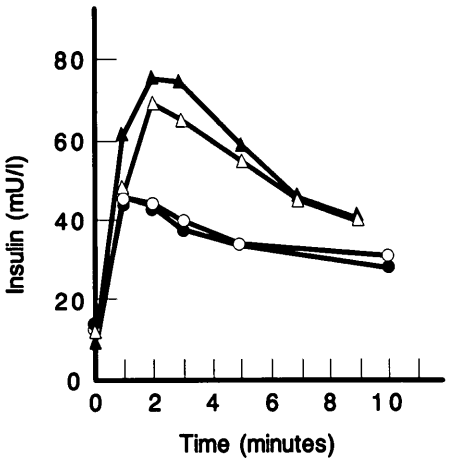

Figure 1—*Mean serum insulin after intravenous glucose in 2 pairs of IVGTTs in 9 subjects. △, Short-infusion test 1; ▲, short-infusion test 2; ○, long-infusion test 1; ●, long-infusion test 2. For ease of comparison, values are superimposed such that the 0 time is designated as the end of each infusion.*

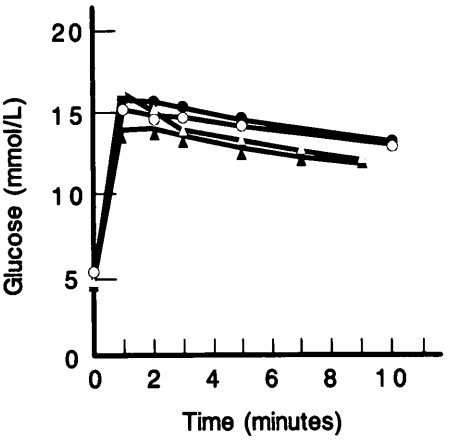

Figure 2—*Mean blood glucose after intravenous glucose in 2 pairs of IVGTTs in 9 subjects. △, Short-infusion test 1; ▲, short-infusion test 2; ○, long-infusion test 1; ●, long-infusion test 2. For ease of comparison, values are superimposed such that the 0 time is designated as the end of each infusion.*

RESULTS

Insulin and glucose profiles in short- and long-infusion tests

Serum insulin in the short-infusion test was significantly higher at 2, 3, and 5 min ($P < 0.05$; Fig. 1). There was no significant difference between mean glucose concentrations in either pair of tests at any time point (Fig. 2). In eight of nine subjects, regardless of FPIR index used, the mean FPIR was significantly higher in the short-infusion than in the long-infusion test.

Variation of the FPIR in the two tests

The mean within-subject CVs for the short-infusion tests compared with long-infusion tests were for 1 + 3-min insulin 18 vs. 16%, 1 + 3 + 5-min insulin 17 vs. 15%, 2 + 3 + 5-min insulin 16 vs. 15%, 0- to 5-min area under the insulin curve 19 vs. 21%, 0- to 10-min area under the insulin curve 18 vs. 19%, and ratio of the 0- to 10-min insulin-to-glucose areas 17 vs. 16%. The range of CVs for each pair of tests was wide, up to 56% in some subjects.

CONCLUSIONS— This study has made two important observations. First, FPIR after the short infusion was greater than after the long infusion. Second, reproducibility was similar for both protocols regardless of the method of analyzing FPIR. In the study of Smith et al. (3), who gave 25 g glucose as a 50% solution over 75 s at 20 g/min, the mean within-subject CVs were slightly higher than those in our study, but the ranges were similarly wide. On the other hand, Rayman et al. (6) reported that within-subject variation of the FPIR could be reduced to <10% by retrograde sampling from an arterialized hand vein. They suggested that slight errors in timing or reduced venous blood flow due to stress-induced vasoconstriction or partial venous constriction by antegrade cannulation could substantially affect reproducibility. However, vasoconstriction due to glucose may not be a major factor; Koschmann et al. (7) showed that the median between-arm CV for 1 + 3-min

insulin concentrations sampled simultaneously from both cubital fossae is only 7.6%. Thus, the lower CV achieved by Rayman et al. (6) may relate to either arterialization and/or the placement of the catheter antegrade. The dose of glucose may also be important. In a separate study, we used a lower dose of glucose (5 g/m^2) injected over 30 s and found even lower within-subject CVs than seen in this study (unpublished observations).

The finding of markedly different acute insulin responses in the two IVGTT protocols emphasizes the difficulty of comparing data between centers with different protocols and the need for each center to establish its own control range. The ICARUS (ICA Register User's Study) working group has recently proposed a new standard protocol (8). This protocol suggests uniform preparation before the test, a glucose dose of 0.5 g/kg up to a maximum of 35 g given as a 25% solution over 3 ± 0.25 min and time zero to be defined as the end of the infusion. We conclude that the reproducibility of the FPIR was not significantly affected by rate of glucose infusion in the two IVGTTs compared and that the difference in FPIR with the different protocols emphasizes the need for standardization of the IVGTT.

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