Standardization of IVGTT to Predict IDDM

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OBJECTIVE — To review current practice in centers that use the IVGTT for prediction of IDDM. To establish consensus protocol for performance of the test.

RESEARCH DESIGN AND METHODS — Postal questionnaires were delivered to 12 centers.

RESULTS — Eleven centers used a glucose dose of 0.5 g/kg and 1 used 0.3 g/kg; the dosage in adults was limited to a maximum of 25–50 g in some centers but others applied no upper limit. The glucose concentration of the infusate varied between 20 and 66%. Eight centers injected glucose manually, two used a syringe pump, and two used gravity infusion. The period of infusion ranged from 30 ± 10 s to 4 ± 2 min, and time zero was taken as the start (1 center), middle (1 center), or end (10 centers) of the infusion. The potential range in timing of the +1-min sample varied between 1 and 7 min from the start of the infusion. Quality-assurance standards for the insulin assays used were not always appropriate for the fasting and low stimulated range of insulin levels.

CONCLUSIONS — The first-phase insulin response to the IVGTT is widely measured as an index of risk of progression to IDDM. We established that methodology varies widely. Because of this, a new standard protocol for use in prediction of IDDM was agreed by an ICARUS working group and is described herein.

Prediction of IDDM is based on measurement of circulating autoantibodies such as ICAs and IAAs together with assessment of the first-phase insulin response to glucose in the IVGTT. Loss of the first-phase response in individuals with high levels of ICA and/or IAA is highly predictive for development of IDDM. Standards for ICA and IAA assay have been established recently, but no agreed standard procedure has been established for IVGTT, although it has been in use for many years. Because of its importance in the prediction of IDDM and the need for comparable methodology for the conduct of multicenter trials of intervention therapy (1), we have surveyed protocols used for IVGTT in numerous centers investigating the pathogenesis and prediction of IDDM and propose a standard protocol for use in future studies in prediabetes.

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IVGTT, INTRAVENOUS GLUCOSE TOLERANCE TEST; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ICARUS, ISLET CELL ANTIBODY REGISTER USER'S STUDY; ICA, ISLET CELL ANTIBODY; IAA, INSULIN AUTOANTIBODY; OGTT, ORAL GLUCOSE TOLERANCE TEST.

RESEARCH DESIGN AND METHODS

— Postal questionnaires were sent to 12 centers that had published or presented work on the use of metabolic tests to predict IDDM. Five were in North America, five in Europe, and two in Australasia. The questionnaires covered details of procedure before testing, dose and rate of glucose infusion, and timing of samples. Sample handling and glucose and insulin assays were also compared, with emphasis on quality assurance.

RESULTS

— All 12 centers responded to the questionnaire.

Preparation

Four centers gave no dietary advice for the days before testing, one center asked subjects to ensure a generous carbohydrate intake on the previous evening, and seven centers specified a high carbohydrate intake (150–300 g) for 3 days before the test. All required a fast of 8–12 h, and the test was started between 0700 and 1000 in all centers.

Glucose infusion

Dose. Eleven of 12 centers used a dose of 0.5 g/kg. Four centers applied no upper limit to the dose, and five centers gave a maximum dose of 25–50 g. One center gave 0.3 g/kg, up to a maximum of 25 g.

Concentration. The final concentration of glucose infused varied between 20 and 66%.

Method of infusion. Eight of 12 centers gave a timed infusion of glucose with a manually driven syringe. A syringe pump

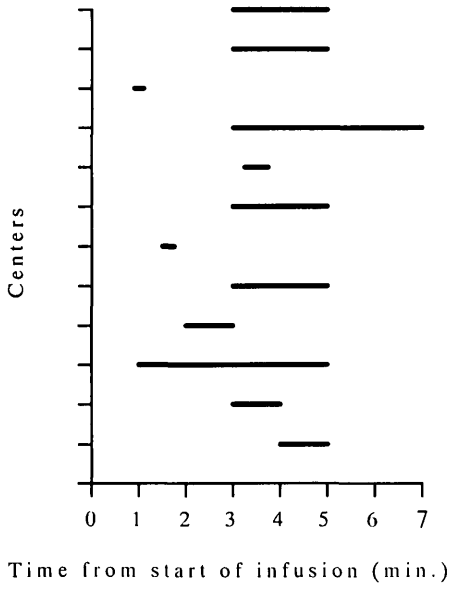

Figure 1—*Potential variation in timing of +1-min sample, with reference to start of glucose infusion. For example, the top-most 2 centers consider time 0 as the end of glucose infusion and allow a time range of 2–4 min for infusion. Consequently, the +1-min sample could represent a time point anywhere between 3 and 5 min from start of infusion, as shown by solid bar.*

was used in two centers, and glucose was delivered by gravity infusion in the remaining two centers.

Duration of infusion. Nine different ranges for accepted infusion time were in use in the 12 centers, varying from 20–40 s to 2–6 min. Four centers stipulated an infusion time of 2–4 min.

Sampling

Designation of time zero. One center designated the beginning, 1 the middle, and 10 the end of the infusion as time zero.

Sampling times. Figure 1 shows the potential range of time lapse between the start of the glucose infusion and the +1-min sample. This ranged from 1 to 7 min between centers. The number of samples taken over the 1st 10 min, excluding baseline samples, ranged from 4 to 7.

Cannulae. A single cannula was inserted and used for both glucose infusion and sampling in 8 of 12 centers; the remainder used two cannulae.

Arterialization. Only one center attempted to arterialize the venous blood by warming the hand with a heated pad.

Table 1—*Range of insulin concentrations covered by quality-assurance material*

Center	Quality-control material (mU/L)	
	Lowest	Highest
1	16	95
2	9.3	30.8
3	6.1	41
4	12.5	43.5
5	8	136
6	9	87.5
7	4	75
8	14.2	32.4
9	40	40
10	8	72
11	29	78
12	Not given	

Glucose assay

Glucose was estimated on plasma or serum samples in 7 of 11 centers providing this information. The remainder measured whole-blood glucose.

Insulin assay

Insulin was measured by radioimmunoassay, with various commercial kits and customized assays. All centers used some form of quality assurance for their assay. This was both external and internal in 10 centers and internal only in two. The range of insulin concentrations covered by the quality-assurance material is shown in Table 1.

CONCLUSIONS— The IVGTT was first used in 1917 by Jorgensen and Plum (2) and developed by Lundbaek, who argued in 1962 that it was the test of choice for the diagnosis of diabetes, because variable gastrointestinal absorption complicates interpretation of OGTT (3). However, it has been used primarily as a research tool, which may explain why it has never been standardized.

Our survey confirmed that methodology for performance of IVGTT varies very widely, so that data cannot usefully be compared or pooled until the test has been standardized. As a result, an expert group appointed by ICARUS developed a consensus protocol (Table 2). Before consensus could be reached, several important factors had to be considered.

Previous diet affects insulin responses to OGTT, and glucose tolerance deteriorates if <125 g carbohydrate is taken daily before the test (4). A minimum of 150 g carbohydrate/day for 3 days is therefore proposed for IVGTT. Physical fitness may affect insulin responses to IVGTT, and unusual exertion should be avoided before the test. Both OGTT and IVGTT show diurnal variation of glucose tolerance and insulin response (5,6), and all tests should begin at the same time of day.

High-risk individuals, many of whom will be children, often need repeated testing. Therefore, the test should be as simple and acceptable as possible. Use of a single catheter to infuse glucose and withdraw samples risks contamination of samples with glucose, but this can be overcome by flushing the line carefully after the glucose is given. The test should be as brief as possible. There is no evidence to suggest that the second-phase response is useful in the prediction of IDDM, so that only the first-phase response needs to be considered. The sum of the insulin concentrations 1 and 3 min after the glucose bolus has been the measure of first-phase insulin release most widely used in the prediction of IDDM, but the 0- to 10-min incremental insulin area may be more reproducible, at least in nondiabetic subjects (7). It also is possible that reproducibility may differ between ICA^+ and control subjects. Therefore, we have suggested that a minimum of four samples should be taken over the 1st 10 min to allow various measures of response to be evaluated.

Table 2—*IVGTT protocol*

PREPARATION: AS RECOMMENDED BY THE NATIONAL DIABETES DATA GROUP FOR OGTTs; I.E., 3 DAYS OF UNRESTRICTED DIET (CONTAINING AT LEAST 150 G CARBOHYDRATE) AND NORMAL PHYSICAL ACTIVITY (13). UNUSUAL PHYSICAL EXERTION SHOULD BE AVOIDED FOR 1 DAY BEFORE TEST. TEST SHOULD BE DEFERRED IF SUBJECT HAS INTERCURRENT ILLNESS.

FAST: AT LEAST 10 H BUT NOT >16 H. WATER IS PERMITTED DURING THIS PERIOD, BUT SUBJECT SHOULD NOT SMOKE.

TIME OF STARTING TEST (GLUCOSE INFUSION): 0730–1000.

GLUCOSE DOSE: 0.5 G/KG UP TO 35 G MAXIMUM.

GLUCOSE CONCENTRATION INFUSED: 25%.

INFUSION: MANUAL OR PUMP-DRIVEN SYRINGE, TIMED TO ENSURE STEADY RATE OF INFUSION.

DURATION OF INFUSION: 3 MIN ± 15 S.

TIME ZERO: END OF INFUSION.

MINIMUM SAMPLES TO BE COLLECTED: 2 BASELINE SAMPLES 5 MIN APART (THE LATTER TAKEN IMMEDIATELY BEFORE GLUCOSE INFUSION) AND +1, +3, +5, +10 MIN AFTER END OF INFUSION.

CANNULA: A SINGLE FOREARM VEIN CANNULA MAY BE USED BUT SHOULD BE FLUSHED WITH SALINE AFTER GLUCOSE IS INFUSED. DEADSPACE SHOULD BE CLEARED BEFORE SAMPLES ARE DRAWN.

IVGTT has poor reproducibility (7), but lower intra-individual coefficients of variation have been reported when the hand has been placed in a water bath at 43°C for 10 min before and during the test (8) or, more simply, under a 60°C, thermostatically controlled heating pad (9). Because this would add somewhat to the complexity of the test, the value of arterializing venous blood and the stringency with which this should be performed needs to be confirmed.

Both the dose and the rate of glucose infusion affect the magnitude of the acute insulin response. A linear relationship between acute insulin response and glucose is observed with doses ranging from 0.5 to 20 g, but higher doses do not increase the response (10). Timing is important also, and comparison of the response to 20 g glucose given over 0.3, 3, 6, and 12 min showed that peak insulin and incremental 0- to 9-min insulin area fall significantly with slower rates of infusion. However responses reached a plateau at infusion rates >7 g/min (11). Rate of infusion may be more important than dose, because doses of 5, 10, and 20 g glucose produced similar insulin responses when given at the same slow rate of infusion (11). Chen and Porte (11) concluded that "For practical purposes, an IVGTT might best be performed using a maximal rate (≥7 g/min), and a maximal dose (≥20 g)."

In our survey, most centers used a maximum dose >20 g and a maximum dose of 35 g was agreed, with a 3-min infusion period; because this procedure will produce maximal stimulation with minimal deviation from established protocols. One advantage of the slower rate of glucose infusion is that it is more comfortable for the recipient, but rapid rates may generate a more-reproducible insulin response, and this possibility is under investigation. One of the most striking differences we revealed was the differing designation of time zero, and standardization is clearly essential.

Insulin assays also must be comparable. A standard textbook of clinical chemistry states that "The concentration of analyte in different control materials should be in the normal and abnormal ranges, corresponding to the concentrations that are critical in the medical interpretation of the test results" (12). Precise determination of the first-phase response requires accurate measurement of both basal and stimulated levels. The assay must be precise at physiological fasting insulin concentrations. Because changes in first-phase response at the lower end of the range are of greatest clinical interest, insulin assays should perform well at the lower end of the stimulated range. Wide variations were revealed by our survey in the lowest quality-control material used, and we would suggest that, as a minimum, one control should be within the normal range for fasting insulin (~5–10 mU/L) and another in the range of low stimulated responses (~20–30 mU/L). Each assay also should be monitored by an external proficiency program; such programs are essential for quality maintenance.

There have been remarkably few attempts to establish a scientific basis for optimum performance of IVGTT, even in normal subjects. The protocol we have proposed is essentially a pragmatic rather than scientific resolution of existing differences between centers and focuses on the first-phase response, because this appears to be of greatest value for the prediction of IDDM. It will be necessary to evaluate this protocol, in normal and high-risk subjects, in terms of its reproducibility and its ability to stimulate a maximal response. A large pool of data from the normal population will also be needed for comparison with data derived from high-risk individuals. There are numerous unresolved issues. Should basal insulin concentrations be subtracted when calculating insulin responses? Can interpretation of insulin secretion data be improved by considering insulin sensitivity? Can the prediction of IDDM be improved by taking account of the effects of age, pubertal status, and body mass index on first-phase response? Can other metabolic tests be used to complement IVGTT? Collaboration of many centers

around the world will allow rapid and full evaluation of the role of IVGTT in predicting IDDM and will standardize measurement of the first-phase insulin response for use in prospective trials of intervention.

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