Reproducibility of the First-Phase Insulin Response to Intravenous Glucose Is Not Improved by Retrograde Cannulation and Arterialization or the Use of a Lower Glucose Dose

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OBJECTIVE — To determine whether the reproducibility of the first-phase insulin response (FPIR) measured during an intravenous glucose tolerance test is improved by the use of a lower glucose dose or retrograde sampling from an arterialized hand vein.

RESEARCH DESIGN AND METHODS — Previous studies have suggested that the high within-subject variation of FPIR measurement of up to 110% could be reduced by sampling from a retrograde cannulated and arterialized hand vein opposite to the cubital fossa vein through which the glucose was injected or by the use of a lower dose of glucose. Two low-dose (glucose, 5 g/m^2 injected over 30 s) and two standard Islet Cell Antibody Registry Users Study (ICARUS) (glucose, 0.5 g/kg injected over 3 min) tests were performed on seven normal subjects at 2-week intervals. Samples were collected simultaneously from the cubital fossa vein, through which the glucose was injected, and from a retrograde cannulated, contralateral hand vein that was arterialized by heating. FPIR was expressed as the sum of the insulin measurements 1 and 3 min after the completion of the glucose injection and as the area under the insulin curve between 0 and 10 min.

RESULTS — Responses to the mean sum of serum insulin concentrations at 1 and 3 min after intravenous glucose were significantly lower for the low-dose test (mean 94 mU/l) than for the high-dose test (mean 184 mU/l) for samples taken from the arm ($P < 0.05$); mean 0- to 10-min insulin areas were 367 and 596 mU/l for low- and high-dose tests, respectively ($P < 0.05$). Within-subject coefficients of variation for samples from the hand or the arm ranged from 0.33 to 17.5% and 1.3 to 38% for successive ICARUS and low-dose tests, respectively. Reproducibility, measured by the coefficient of variation between successive tests for each protocol, was not significantly different using samples taken from the arm or the contralateral hand.

CONCLUSIONS — The intravenous glucose tolerance test is reproducible when performed by the same operator over a short time span. Reproducibility is not significantly improved by sampling from an arterialized, retrograde cannulated, contralateral hand vein. There is no case for changing the present ICARUS protocol to incorporate retrograde cannulation or low-dose (5 g/m^2) glucose.

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CV, coefficient of variation; FPIR, first-phase insulin response; ICARUS, Islet Cell Antibody Registry Users Study; IVGTT, intravenous glucose tolerance test.

The measurement of first-phase insulin response (FPIR) to glucose during an intravenous glucose tolerance test (IVGTT) is used in islet cell antibody-positive subjects to assess β-cell reserve. An FPIR less than the first percentile for normal subjects is highly predictive of the onset of clinical type I diabetes within 3 years (1,2). Srikanta et al. (3) proposed that there was a linear decline in FPIR before the onset of type I diabetes in islet cell antibody-positive relatives. However, other studies (4) have demonstrated that the within-subject variation of the FPIR in an IVGTT can vary between 1 and 110%, casting doubt on the ability of the test to detect minor changes in FPIR. This poor reproducibility is most likely due to a combination of factors that can affect insulin responses to both oral and intravenous glucose. These include diet (5), ambient temperature (6), diurnal variation (7,8), and pubertal status (9). Psychological stress has also been reported to reduce basal insulin measurements (10) and may have a significant effect on glucose-stimulated insulin secretion. In addition, factors specific to the test such as the rate of glucose injection and the total dose of glucose administered have major influences on FPIR (11,12). These factors and the finding of a recent survey that nearly all centers performing the IVGTT were using differing protocols for the test led the Islet Cell Antibody Registry Users Study (ICARUS) to introduce a recommended standard protocol (13). However, even with such standardization, the high within-subject variation and inability to demonstrate small changes of FPIR within the very wide normal range remains a problem (14). As intervention therapy in preclinical diabetes becomes a reality, the ability to measure β-cell reserve reliably is critical. Thus, it is essential to determine whether the within-subject variation of FPIR measurement can be reduced to a level such that an authentic reduction within the normal range can be reliably identified.

Recently, several modifications to the standard IVGTT protocol have been reported to reduce within-subject variation of the FPIR. First, Rayman et al. (15) reported improved reproducibility in normal subjects when blood samples were taken from an arterialized vein cannulated retrogradely in the back of the contralateral hand rather than from the antecubital vein through which glucose was administered. In contrast, Rowe et al. (16) found that sampling from a retrogradely cannulated hand vein held no advantage over conventional arm vein cannulation. Second, Tan et al. (17) found improved reproducibility in normal subjects when a low dose of glucose (5 g/m^2 body surface area) was administered over 30 s. These modified protocols have not as yet been compared with the standard IVGTT protocol.

In this study, we compared the reproducibility of measuring FPIR in normal subjects in an IVGTT using a low dose of glucose (5 g/m^2 body surface area) given over 30 s and in an IVGTT using the recommended ICARUS protocol. Furthermore, in each protocol, the effect of sampling from an arterialized, retrogradely cannulated contralateral hand vein, as proposed by Rayman et al. (15), was compared with sampling from the antecubital vein through which the dose of glucose was administered.

RESEARCH DESIGN AND METHODS

— Fourteen subjects (5 men and 9 women aged between 19 and 23 years) with no family history of type I diabetes were studied. Subjects provided written, informed consent before taking part in the study, which was approved by the Royal Melbourne Hospital Ethics Committee. All subjects were negative for islet cell antibodies and insulin autoantibodies and had normal glycated hemoglobin levels. Each subject underwent two low-dose and two ICARUS-recommended standard protocol IVGTTs in random order at intervals of 2 weeks. Subjects fasted for 10–14 h before each test, abstained from smoking, and avoided unusual physical exertion for 1 day before the test. Subjects were instructed to maintain their normal dietary intake and to include at least 150 g carbohydrate per day in the 3 days before the test. Local anesthetic cream (EMLA, Astra, North Ryde, Australia) was offered to subjects to reduce both the discomfort involved in retrograde cannulation of the hand and anticipation anxiety.

Procedure

For 15 min before and throughout the IVGTTs, the subject's hand, opposite to the arm through which the glucose was administered, was warmed with an electric blanket (Assist-Heat Sleeve, Breville, Pyrmont, Australia). In each test, blood samples were collected simultaneously by two registered nurses, from a cannula inserted into a cubital fossa vein (through which the glucose dose was injected) and from a cannula inserted retrogradely into a vein in the back of the contralateral hand. In four subjects, each of whom completed all four tests, blood was taken simultaneously from both sites for blood gas measurement before the glucose injection.

In the low-dose protocol, glucose, 5 g/m^2 body surface area as a 25% solution, was injected manually over 30 s into an antecubital vein. In the ICARUS protocol, glucose, 0.5 g/kg body weight as a 25% solution (up to a maximum of 35 g), was injected manually over 3 min into an antecubital vein. For each subject, the same nurse took the hand samples in all of the tests, while the other nurse took all of the arm samples.

FPIR analysis

The end of the injection was taken as time zero for FPIR analysis. FPIR was calculated either as the sum of the serum insulin concentrations at 1 and 3 min or as the area under the insulin curve (with basal insulin subtracted) from 0 to 10 min.

Serum insulin was measured using a commercial RIA kit (Pharmacia, Uppsala, Sweden). All samples from each subject were analyzed in the same assay. The interassay coefficient of variation (CV) was 7.3% at low (8.5 mU/l), 4.3% at medium (28 mU/l), and 3.5% at high (90 mU/l) insulin concentrations. Islet cell antibodies and insulin autoantibodies were assayed using internationally standardized immunofluorescence and radiobinding assays, respectively. Glycated hemoglobin was measured using an affinity-chromatography method on a Column Mate instrument (Helena, Beaumont, TX) (nondiabetes range <7%).

Statistical analysis

CVs were used to compare the test methods. They were calculated using an SD for normally distributed samples as

$$SD_{pair} = \sqrt{\frac{\sum_1^2 (x - \bar{x}_{pair})^2}{n - 1}}, \quad CV = \frac{SD_{pair}}{\bar{x}_{pair}} \times 100$$

Comparisons of FPIRs and CVs between protocols and between sample sites were performed using the Mann-Whitney *U* test for nonparametric data (Minitab release 8, Minitab, State City, PA).

RESULTS

— All 4 IVGTTs were completed on 7 (5 men and 2 women) of the 14 subjects. Results for the seven completed sets of ICARUS tests are summarized in Table 1. Five subjects had at least one successful IVGTT and two had no successful tests. Failure to cannulate the hand vein retrogradely in a manner allowing adequate blood flow for sampling was the reason for all of the unsuccessful tests. Many subjects found retrograde cannulation painful despite the use of local anesthetic cream.

In all but one of the seven subjects who completed the four tests, the FPIR (1- + 3-min insulin or 0- to 10-min insulin area) was greater in the ICARUS IVGTT than in the low-dose IVGTT (Figs. 1 and 2). The mean 1- + 3-min serum insulin concentration for samples taken from the arm was 94 mU/l for the low-dose test compared with 184 mU/l for the ICARUS test ($P < 0.05$); mean 0- to 10-min insulin areas were 367 and 596 mU/l,

Table 1—*Serum insulin concentrations (mU/l) 1 and 3 min after the glucose infusion and areas under the insulin curve (0–10 min) for the seven subjects*

	1 min insulin (mU/l)				3 min insulin (mU/l)				0–10 min insulin area (mU · min · l^{-1})			
	Arm		Hand		Arm		Hand		Arm		Hand	
Subject	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	93	64	110	82	78	56	68	68	454	353	442	414
2	310	240	270	240	230	200	240	190	2025	1474	1843	1411
3	79	73	78	75	62	51	50	61	461	331	361	427
4	88	77	96	88	61	71	66	51	383	412	393	390
5	57	67	73	58	51	52	61	50	342	373	404	344
6	82	86	100	100	47	75	80	71	427	489	517	452
7	51	75	56	54	56	45	50	48	400	424	312	322

Each subject completed two ICARUS protocol IVGTTs with samples being taken simultaneously from the arm through which the glucose was injected and from a retrogradely cannulated vein in the back of the warmed contralateral hand.

respectively ($P < 0.05$). The mean 1- + 3-min serum insulin concentration for samples taken from the hand was 114 mU/l for the low-dose test compared with 188 mU/l for the ICARUS test ($P < 0.05$); mean 0- to 10-min insulin areas were 407 and 574 mU/l, respectively ($P < 0.05$).

Within-subject CVs for 1- + 3-min insulin with the ICARUS test ranged from 0.33 to 17.5% (mean 8.0%) for samples taken from the cubital fossa vein and 1.9 to 10.7% (mean 6.1%) for samples taken from the hand vein ($P > 0.05$). With the low-dose test, the ranges were 5.2–19.2% (mean 11.4%) and 1.3–38.0% (mean 19.0%) for samples taken from the cubital fossa vein and the hand vein, respectively ($P > 0.05$). The CVs for 1- + 3-min insulin for the ICARUS test were not significantly lower than those in the low-dose test for sampling from the hand vein ($P < 0.05$) or from the cubital fossa vein ($P > 0.05$) (Fig. 3).

CVs for 0- to 10-min insulin areas with the ICARUS test ranged from 2.9 to 15.7% (mean 8.9%) for samples from the cubital fossa vein and 1.6 to 13.2% (mean 6.0%) for samples from the hand vein ($P > 0.05$). With the low-dose test, ranges were 3.2–32.5% (mean 13.8%) and 2.0–22.4% (mean 12.5%) for samples taken from the cubital fossa vein and the hand vein, respectively ($P > 0.05$). The CVs for 0- to 10-min insulin areas for the ICARUS test were not significantly different from those for the low-dose test with samples from either the cubital fossa ($P > 0.05$) or the hand vein ($P > 0.05$) (Fig. 4).

Variation of insulin measurement on samples taken from the two different sites at identical times was calculated for all of the ICARUS tests. Mean CVs were 5.8% (range 3.7–9.8%) for the 1- + 3-min insulin and 6.9% (range 2.1–13.0%) for the 0- to 10-min insulin area.

For comparison with the results by Rayman et al. (15), median within-subject CVs for the 1-min insulin measure in successive ICARUS tests were calculated for samples taken from both the hand and the arm. Median CVs were 8.1% (range 2.4–19.0%) for samples taken from the arm and 4.3% (range 0–14.5%) for samples taken from the hand.

Partial pressure of oxygen was higher in all samples taken from the hand

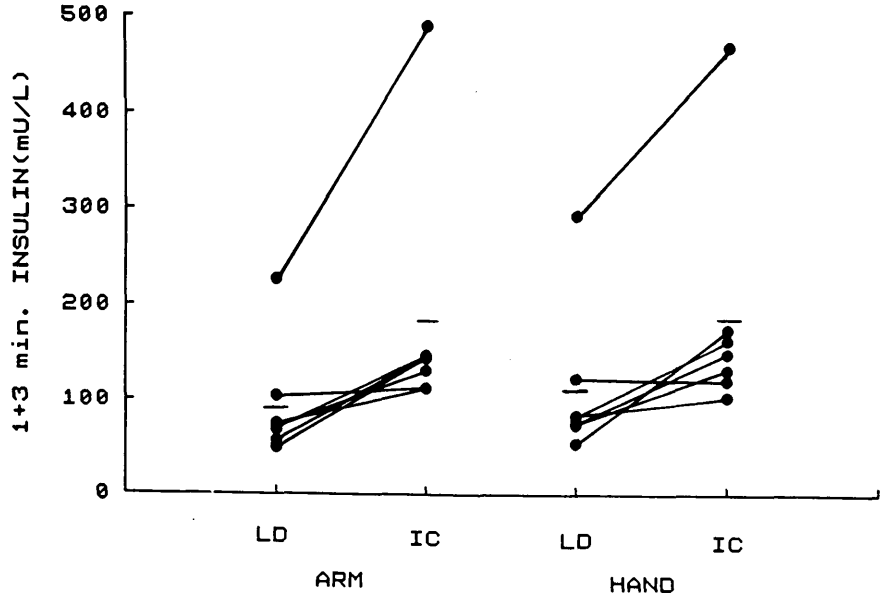

Figure 1—*Mean FPIR responses (calculated as the sum of the serum insulin concentrations at 1 and 3 min after intravenous glucose) in seven normal subjects. Comparison between the low-dose glucose (LD) and ICARUS (IC) IVGTT protocols with sampling from a vein in the antecubital fossa through which the glucose was administered (ARM) or from a retrogradely cannulated vein on the back of the contralateral hand (HAND). In all figures, group means are indicated by the horizontal lines.*

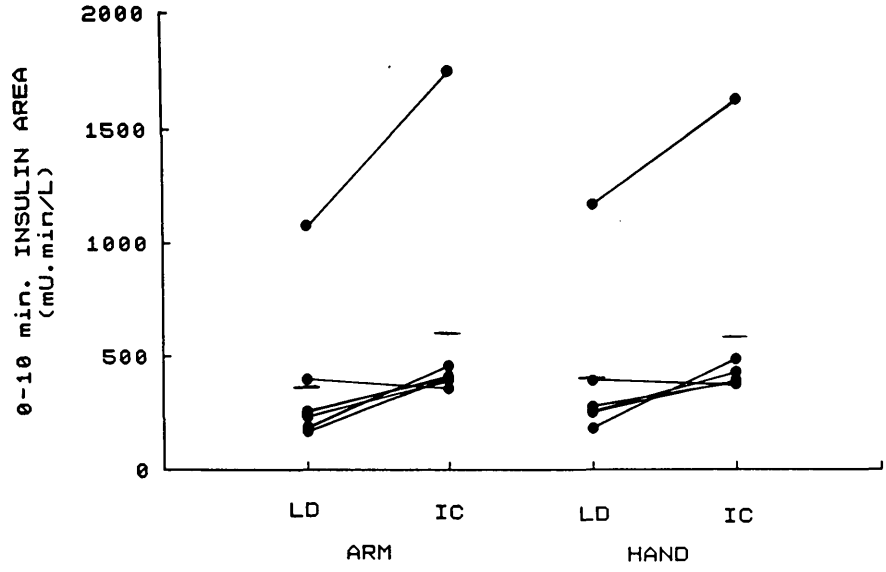

Figure 2—*Mean FPIR responses calculated as the area under the serum insulin curve between 0 and 10 min after intravenous glucose (basal insulin concentrations subtracted) in seven normal subjects. Comparisons as described in Fig. 1.*

vein compared with those from the antecubital vein (Fig. 5), the mean Po_2 levels being 79 and 49 mmHg, respectively ($P < 0.01$).

CONCLUSIONS — This study demonstrates that the FPIR to glucose in the IVGTT using the ICARUS-recommended standard protocol is reproducible in normal control subjects when performed by experienced operators. Moreover, a lower glucose dose or sampling retrogradely from an arterialized vein on the back of the warmed hand did not lead to a significant improvement in reproducibility of FPIR measurement.

Retrograde cannulation was successfully achieved in only 7 of 14 subjects. In these subjects, Po_2 levels were consistently higher in the warmed hand, indicating that at least partial arterialization was achieved. However, retrograde cannulation of the hand vein was often painful despite the use of local anesthetic cream. In prediabetes studies, many of the subjects undergoing IVGTTs are children, in whom the requirement for two sites of venous access and the pain associated with retrograde cannulation would, in any case, make this approach unacceptable.

Use of the same venous access for both glucose injection and blood sampling is the most common approach in the IVGTT. The mean CV for the 1- + 3-min FPIR between consecutive IVGTTs with the ICARUS protocol and a single access site was 8.0% (range 0.33–17.5%); the CV for the 0- to 10-min insulin area was similar. Because the intra-assay CV for the insulin assay is 3–5%, the mean variation attributable to the IVGTT technique and other factors may be as low as 5%. This is supported by the mean within-test variation between samples taken simultaneously from the arm vein and the contralateral hand vein of <7% (range 2.1–13.0%) for the ICARUS tests. If this is a true indication of the mean variation between sampling sites within a single test, it is unlikely that this variation will be improved between successive tests. Indeed, in this study the CVs for successive ICARUS tests vary by up to 18% from subject to subject. Thus, the detection of small changes in FPIR within an individual remains a problem.

Smith et al. (4) achieved a median within-subject CV of 22% (range 3–55%) for the 0- to 10-min insulin area. Comparison between the CVs achieved in this study and those of Smith et al. (4) for the

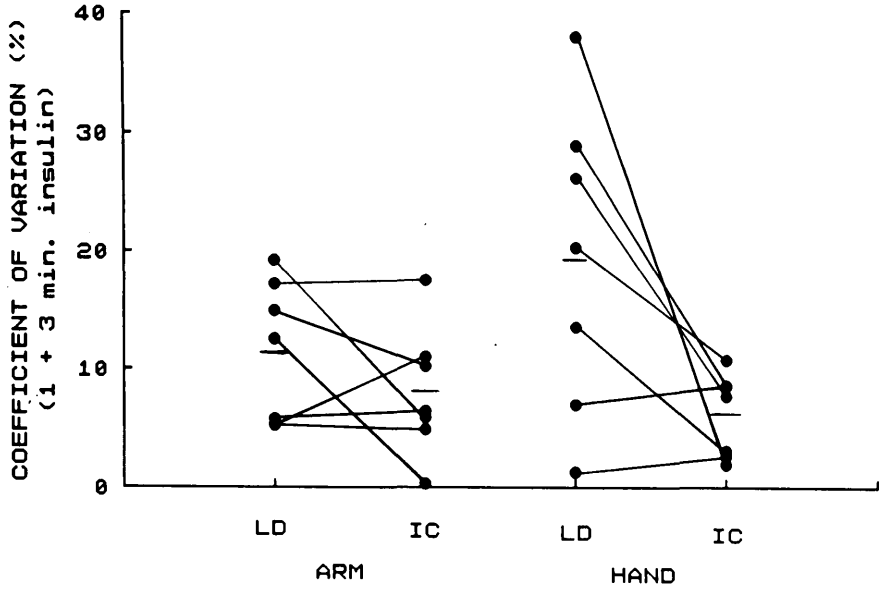

Figure 3—*Mean CV calculated as the SD/mean for the sum of the serum insulin concentrations at 1 and 3 min after intravenous glucose for two consecutive tests in seven normal subjects. Comparisons as described in Fig. 1.*

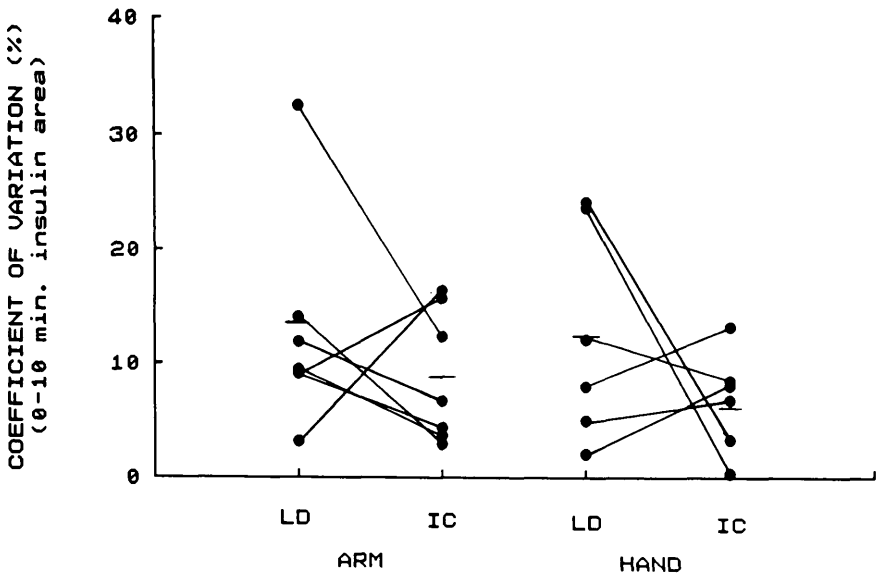

Figure 4—*Mean CV calculated as the SD for the area under the serum insulin curve between 0 and 10 min after intravenous glucose (basal insulin concentrations subtracted) for consecutive tests in seven normal subjects. Comparisons as described in Fig. 1.*

0- to 10-min insulin area showed a significant difference for samples taken from the hand site ($P < 0.05$) but not for the arm site ($P > 0.05$) in the ICARUS test. Raw data for the 1- + 3-min insulin measures were not published by Smith et al. (4), but median coefficients of variation in this study appear to be lower than those reported.

The FPIR was significantly higher in the ICARUS than in the low-dose tests. This indicates that in many subjects, the low-dose test elicits a submaximal response. In the low-dose test, the amount of glucose and the rate of administration was approximately 10 and 20 g/min, compared with 35 and 11.7 g/min for the ICARUS test. Chen and Porte (12) reported that a dose of at least 20 g of glucose at a rate of >7 g/min is required to obtain a maximal FPIR in adults. Thus, both tests exceeded the minimum rate requirement. However, the total dose in the low-dose tests may have been below that required for a maximal response. It remains to be shown whether the concentration of hormone released per unit of glucose in low-dose tests is a more sensitive marker in subjects with β-cell damage.

Rayman et al. (15) reported improved reproducibility when samples were taken from an arterialized vein in the back of the contralateral hand. Within-subject reproducibility in the study of Rayman et al. was expressed as a median CV of 4.0% (range, 1.2–24.3%) for the 1-min insulin (3 min after the commencement of the injection) and 6.7% (range, 1.7%–18.8%) for the 0- to 10-min insulin area. In the current study, median CVs in the ICARUS tests for samples taken from the hand vein were 4.3% (range 0–14.5%) for the 1-min sample, 7.6% (range 1.9–10.7%) for the 1- + 3-min calculation, and 6.75% (range 0.37–13.2%) for the 0- to 10-min area. These results are similar to the median CVs for samples taken from the arm vein through which the glucose was injected: 8.1% (range 2.3–19.0%) for the 1-min sample; 6.41% (range 0.33–17.5%) for the 1- + 3-min measure; and 6.73% (range 2.92–16.4%) for the 0- to 10-min area. There was no significant difference ($P > 0.05$) between CVs achieved by Rayman et al. (15) and either sampling site in this study for the 1-min insulin measurement or the 0- to 10-min insulin area. Hence, reproducibility was not improved by sampling from the arterialized, retrograde cannulated, contralateral hand in this study or in comparison with results from the Rayman study.

In a recent study, Rowe et al. (16)

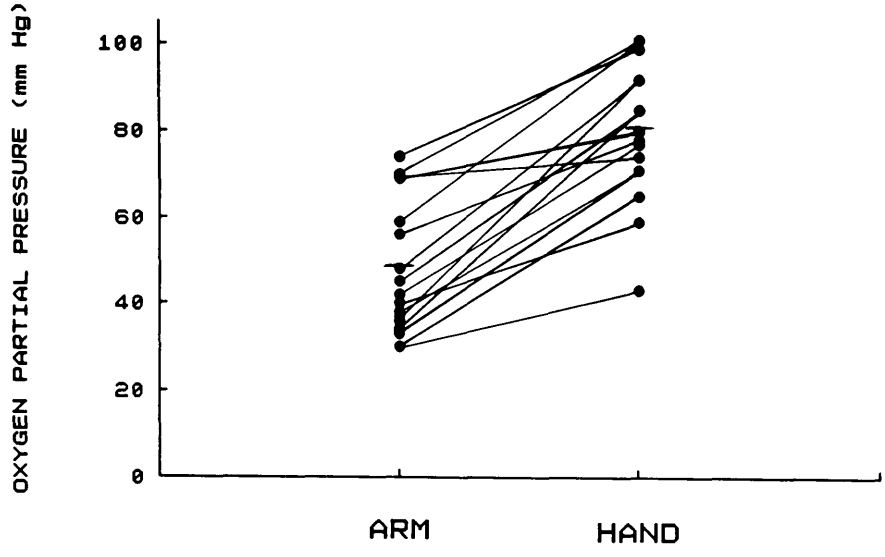

Figure 5—*Partial pressure of oxygen in blood samples withdrawn simultaneously from a cannula in an antecubital vein and from a retrogradely cannulated vein in the contralateral hand (four tests on each of four subjects).*

also compared sampling from a retrogradely cannulated contralateral hand vein and a cubital fossa vein contralateral with that through which the glucose was administered over a series of three tests. FPIR was calculated as the mean insulin measure of samples obtained at 1, 2, 3, 4, 5, 6, 7, and 10 min after the glucose infusion minus the mean of the fasting insulin samples. The CVs for the mean acute insulin response to glucose calculated by Rowe et al. for the retrograde cannulated hand vein (21.5% [range 1.3–39.6%]) and the contralateral cubital fossa vein (22.5% [range 4.0–46.6%]) appear to be higher than those reported in the present study. However, as in this study, Rowe et al. concluded that, in contrast to the study of Rayman et al. (15), there was no difference in the intertest CVs between the two sampling sites.

The measurement of FPIR by the IVGTT in normal subjects, performed by the same operator with the ICARUS protocol, is reproducible within a short time span (<8 weeks). Arterialization and retrograde cannulation of the sampling site does not improve reproducibility. There is no case for changing the present ICARUS protocol to incorporate retrograde cannulation or low-dose (5 g/m^2) glucose.

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References

1. Srikanta S, Ganda OP, Rabizadeh A, Soeldner JS, Eisenbarth GS: First degree relatives of patients with type 1 diabetes mellitus: islet cell antibodies and abnormal insulin secretion. *N Engl J Med* 313: 461–464, 1985
2. Vardi P, Crisa L, Jackson RA: Predictive value of intravenous glucose tolerance test insulin secretion less than or greater than first percentile in islet cell antibody positive relatives of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 34:93–102, 1991
3. Srikanta S, Ganda OP, Gleason RE, Jackson RA, Soeldner JS, Eisenbarth GS: Pretype 1 diabetes: linear loss of β-cell response to intravenous glucose. *Diabetes* 33:717–720, 1984
4. Smith CP, Tarn A, Thomas JM, Overkamp D, Coracki A, Savage MO and Gale EAM: Between and within subject variation of the first phase insulin response to intravenous glucose. *Diabetologia* 31:123–125, 1988
5. West K: *Epidemiology of Diabetes and Its Vascular Lesions*. New York, Elsevier, 1978
6. Akanji AO, Oputa RN: The effect of ambient temperature on glucose tolerance. *Diabetic Med* 8:946–948, 1991
7. Bowen AJ, Reeves RL: Diurnal variation in glucose tolerance. *Arch Intern Med* 119: 261–264, 1967
8. Carroll K, Nestell PJ: Diurnal variation in glucose tolerance and in insulin secretion in man. *Diabetes* 22:333–346, 1973
9. Smith CP, Williams AJK, Thomas JM, Archibald HR, Algar VD, Bottazzo GF, Gale EAM, Savage MO: The pattern of basal and stimulated insulin responses to intravenous glucose in first degree relatives of type 1 (insulin-dependent) diabetic children and unrelated adults aged 5 to 50 years. *Diabetologia* 31:430–434, 1988
10. Kemmer FW, Bisping R, Steingruber HJ, Baar H, Hardtmann F, Schlaghecke R, Berger M: Psychological stress and metabolic control in patients with type 1 diabetes mellitus. *N Engl J Med* 314:1078–1084, 1986
11. Colman PG, Stewart V, Kean J, Koschmann M, Alford F, Ward G, Deam D, Harrison LC: Comparison of two commonly used IVGTTs. *Diabetes Care* 15: 1053–1055, 1992
12. Chen M, Porte D: The effect of rate and dose of glucose infusion on the acute insulin response in man. *J Clin Endocrinol Metab* 42:1168–1175, 1976
13. Bingley PJ, Colman PG, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EAM: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313–1316, 1992
14. McCulloch DK, Bingley PJ, Colman PG, Jackson RA, Gale EAM, ICARUS Group: Comparison of bolus and infusion protocols for determining acute insulin response to intravenous glucose in normal humans. *Diabetes Care* 16:911–915, 1993
15. Rayman G, Clark P, Schneider AE, Hales CN: The first phase insulin response to intravenous glucose is highly reproducible. *Diabetologia* 33:631–634, 1990
16. Rowe RE, Leech NJ, Finegood DT, McCulloch DK: Retrograde versus antegrade cannulation in the intravenous glucose tolerance test. *Diabetes Res Clin Pract* 25: 131–136, 1994
17. Tan KT, Fairley S, Jennens ID, Harrison LC: Low dose vs. conventional dose glucose for intravenous glucose tolerance testing (Abstract). *J Autoimmunity* 3:98, 1990