

# Reduced Sampling Protocols in Estimation of Insulin Sensitivity and Glucose Effectiveness Using the Minimal Model in NIDDM

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Recent work in healthy subjects, the aged, and subjects with gestational diabetes or drug-induced insulin resistance using minimal model analysis of the tolbutamide-modified frequently sampled intravenous glucose tolerance test suggested that a reduced sampling regimen of 12 time points produced unbiased and generally acceptable estimates of insulin sensitivity ( $S_I$ ) and glucose effectiveness ( $S_G$ ) compared with a full sampling schedule of 30 time points. We have used data from 26 insulin-modified frequently sampled intravenous glucose tolerance tests in 21 subjects with NIDDM to derive and compare estimates of  $S_I$  and  $S_G$  from the full sampling schedule ( $S_{I(30)}$ ,  $S_{G(30)}$ ) with those estimated from the suggested 12 time points ( $S_{I(12)}$ ,  $S_{G(12)}$ ) and those estimated with the addition of a 25-min time point ( $S_{I(13)}$ ,  $S_{G(13)}$ ). Percentage relative errors were calculated relative to the corresponding 30 time-point values. A statistically significant bias of 15% (97% confidence interval from 7.4 to 25.6%, interquartile range 25%) was introduced by the estimation of  $S_{I(12)}$  but not  $S_{I(13)}$  (1%, 97% confidence interval from -9.4 to 9.3%, interquartile range 21%). Results for  $S_{G(12)}$  (-12%, 97% confidence interval from -46.7 to 1.2%, interquartile range 49%) and  $S_{G(13)}$  (-5%, 97% confidence interval from -27.8 to 6.8%, interquartile range 37%) were statistically equivocal. The precision of estimation of  $S_{I(12)}$ ,  $S_{G(12)}$ , and  $S_{G(13)}$  measured by the interquartile range of the

percentage relative errors was poor. The precision of determination measured by the median minimal model coefficient of variation was 18, 29, and 27% for  $S_{I(30)}$ ,  $S_{I(12)}$ , and  $S_{I(13)}$  and 9, 11, and 11% for  $S_{G(30)}$ ,  $S_{G(12)}$ , and  $S_{G(13)}$ , respectively. Thus, the application of minimal model analysis to the 12 time-point protocol of the insulin-modified IVGTT for the estimation of  $S_I$  and  $S_G$  in NIDDM may necessitate an inordinately large number of subjects. Although the 13 time-point protocol may be more acceptable for the assessment of  $S_I$  in population studies, we recommend retention of the full sampling schedule where feasible. *Diabetes* 42:1635-41, 1993

The minimal model analysis of the FSIVGTT devised by Bergman et al. (1) derives estimates of both insulin-mediated glucose disposal ( $S_I$ ) and the ability of glucose to restore its own concentration independent of a change in insulin regardless of the prevailing glycemia ( $S_G$ ). This approach has been used to examine alterations in  $S_I$  in subjects with a variety of physiological and pathophysiological states such as in the elderly (2), normotensive subjects with a family history of hypertension (3), obese subjects (4), and those receiving anabolic steroids (5) or oral contraceptive agents (6). The euglycemic-hyperinsulinemic clamp is, however, widely regarded as a reference method for this type of investigation (7). Theoretical considerations aside, the clamp technique is labor intensive for both subject and investigator and as such is unsuitable for widespread use in the investigation of defects of insulin sensitivity. In comparison, minimal model analysis of the FSIVGTT is a relatively simple procedure to carry out (8). For NIDDM, where reduced  $S_I$  is of major pathophysiological significance (9), its use has been restricted by the requirement of the model for an adequate insulin response during the test procedure. Additionally, the number of blood samples required (usually ~30) is high.

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Received for publication 25 March 1993 and accepted in revised form 17 June 1993.

NIDDM, non-insulin-dependent diabetes mellitus; IVGTT, intravenous glucose tolerance test; FSIVGTT, frequently sampled intravenous glucose tolerance test;  $S_I$ , insulin sensitivity;  $S_G$ , glucose effectiveness; MINMOD, minimal model; CV, coefficient of variation; %RE, percentage relative error; IQR, interquartile range; CI, confidence interval; HOMA, homeostasis model assessment; CIGMA, continuous infusion of glucose with model assessment; SIT, short insulin tolerance test; BSA, body surface area; BMI, body mass index.

TABLE 1  
Subject characteristics at initial FSIVGTT

M/F Age (yr)	56.4 ± 8.8 (39–74)
HbA <sub>1c</sub> (%)	10.4 ± 2.28 (7.1–14.0)
BMI (kg/m <sup>2</sup> )	28.38 ± 3.75 (21.8–34.4)
Fasting plasma glucose (mM)	11.45 ± 3.40 (6.3–19.1)

Data are means ± SD (range).

Modification of the FSIVGTT technique with the use of intravenous tolbutamide 20 min after the glucose bolus produces a substantial peak plasma concentration of insulin in normal subjects aiding both the disposal of glucose and the accuracy of subsequent modeling. Reasonable agreement has been claimed between estimates of  $S_I$  derived from the tolbutamide-modified FSIVGTT and the euglycemic-hyperinsulinemic clamp in both normal weight and obese nondiabetic subjects (8,10).

As a result of the unpredictable insulin response to intravenous tolbutamide of many subjects with NIDDM, the use of an intravenous bolus of soluble insulin has been suggested (11). In normal healthy subjects, this insulin-modified FSIVGTT has been shown to result in similar estimates of  $S_I$  to those from tolbutamide-modified tests (11,12). Recently, Steil et al. (13) suggested that the number of blood samples required for estimation of  $S_I$  and  $S_G$  in healthy subjects, the aged, and subjects with gestational diabetes or drug-induced insulin resistance can be reduced from the original 32 to 12 with a minor loss of precision, making the technique useful for population studies. As a result of this suggestion, we have examined data from 26 insulin-modified FSIVGTTs using the full sampling schedule carried out in subjects with established NIDDM. This study aimed to determine whether the above-mentioned reduced sampling regimen could produce estimates of  $S_I$  and  $S_G$  that approximate those estimates derived from the full sampling schedule or whether, as intimated by Steil and Bergman (14) and Bergman et al. (15), additional modification of the timings of the reduced sampling regimen could reduce bias and improve on this precision.

**RESEARCH DESIGN AND METHODS**

After local ethical committee approval and informed consent, 21 normotensive Caucasian subjects (16 men and 5 women) were recruited from the Diabetic Clinic at the University Hospital of Wales. All subjects were either newly presenting and previously untreated (9 men and 4 women) or were treated by diet alone (7 men and 1 woman). All were free of specific diabetes-related complications. Five subjects (4 men and 1 woman) had repeat tests 3 or 6 mo later, and their indicators of metabolic control were sufficiently different to regard the repeat tests as independent of the original tests, thus making a total data set of 26 full sampling schedule insulin-modified FSIVGTTs. Table 1 gives a summary of subject characteristics.

The insulin-modified FSIVGTT took place after a 10-h overnight fast. An intravenous cannula was placed in an antecubital fossa vein of each arm and maintained patent

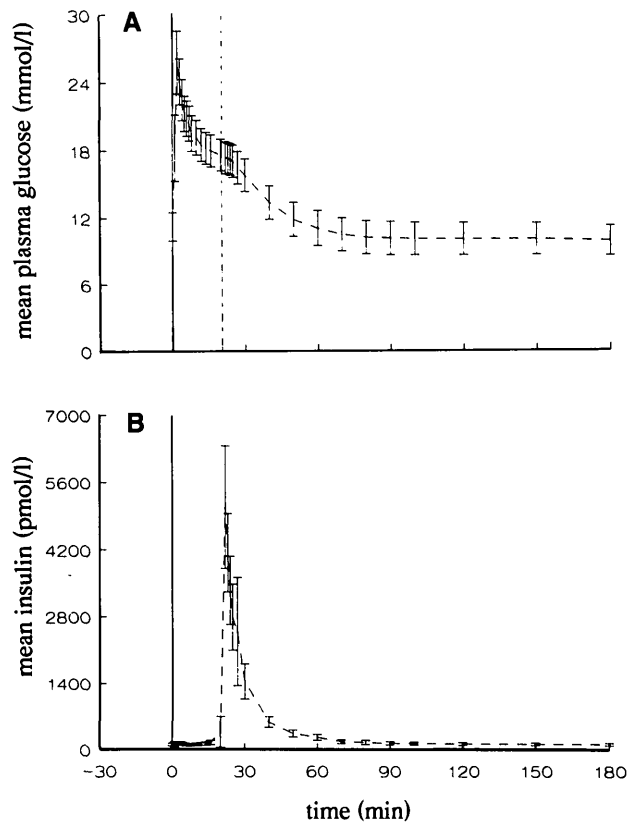
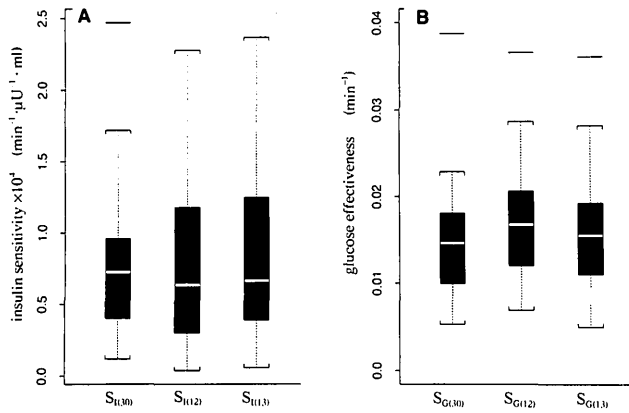


FIG. 1. Mean plasma glucose (A) and insulin (B) profiles. Glucose (300 mg/kg) was infused intravenously at time 0 min followed by a bolus of 0.05 U/kg soluble insulin at time 20 min. Bars represent 95% CIs of means. Individual profiles were consistent with the mean profile. Dashed lines connecting mean values may not represent actual values ( $n = 26$ )

by a slow running infusion of 0.9% saline. After 15 min of rest, basal blood samples were taken at -30, -15, and 0 min. Immediately after the 0-min sample, 300 mg/kg of glucose was infused intravenously at a constant rate over 2 min into the contralateral cannula, followed at time 20 min by a bolus intravenous injection of 0.05 U/kg Human Actrapid (Novo Nordisk, Bagsvaerd, Denmark). Sampling for glucose and insulin took place during the basal period and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min. The samples were separated in a refrigerated centrifuge and stored at -20°C until subsequent assay.

**Analytical methods.** Plasma glucose was assayed by an autoanalyzer (Chemlab, Hornchurch, Essex, UK) with the use of an enzymatic colorimetric method with intra- and interassay CVs of <2%. Immunoreactive insulin was assayed with the use of a modification of the technique of Heding (16) with intra- and interassay CVs of 4.6 and 7.3%, respectively. Glycosylated hemoglobin was assayed by column chromatography with the use of standard reagents (Test-combination HbA<sub>1c</sub>, BCL, Lewes, Sussex, UK).

**MINMOD analysis.**  $S_I$  and  $S_G$  (full and reduced sampling schedules) with corresponding CVs were calculated from the FSIVGTT data with the use of the MINMOD program



**FIG. 2. A:** Distributions of  $S_I$  data. The white line inside each box represents median value; the height of the box gives the IQR; lines outside the “whiskers” denote outliers ( $n = 26$ ). **B:** Distributions of  $S_G$  data ( $n = 26$ ).

(courtesy of R.N. Bergman) with weighting of data as recommended by Pacini and Bergman (17). Sample times used were 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min to give  $S_{I(30)}$  and  $S_{G(30)}$  and, as suggested by Steil et al. (13), 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min to give  $S_{I(12)}$  and  $S_{G(12)}$ .  $S_{I(13)}$  and  $S_{G(13)}$  were determined after the inclusion of the 25-min time point. In all cases, the 0 value was calculated as the mean of the basal samples.

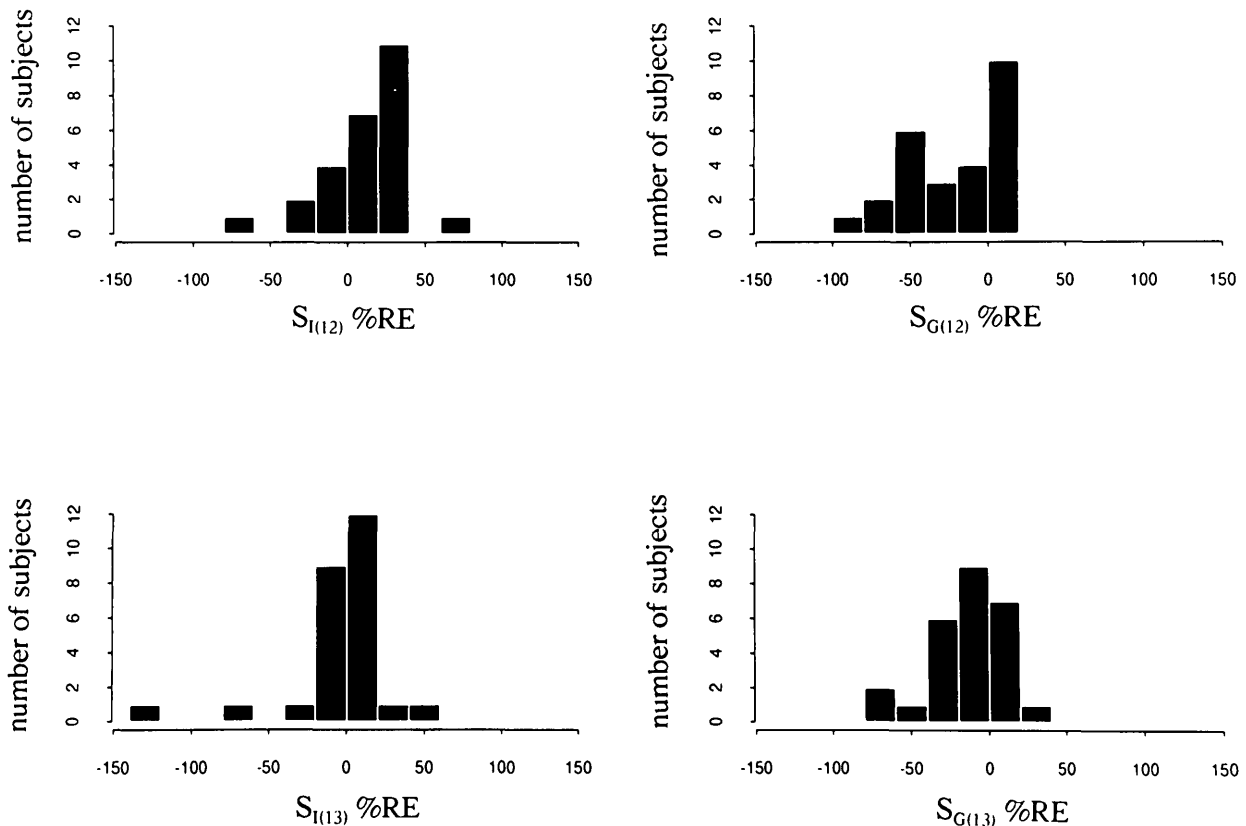
**Statistical analysis.** A study size calculation was conducted based on the following paired values:  $\alpha = 0.01$  (probability of null hypothesis rejection error),  $\beta = 0.15$  (probability of null hypothesis acceptance error), and  $\sigma = 20$  (estimated SD of %RE) as in Steil et al. (13) and  $\Delta = 15$  (minimum %RE required to detect). On this basis, a study size of 26 was used.

%REs of  $S_I$  and  $S_G$  for the reduced sampling regimens versus the full sampling schedule were calculated according to  $100(\text{full} - \text{reduced})/(\text{full})$ .

Boxplots, histograms, and quantile-quantile plots were used to assess the normality of the %RE data. Study size, skewed distributions, and the presence of outliers necessitated the use of a nonparametric statistical approach.

The reduced sampling protocols were assessed in terms of bias (the presence of systematic error), precision of estimation (the level of agreement with the full sampling protocol values), and precision of determination (the size of the CVs).

Wilcoxon’s signed rank test was used to evaluate the statistical support for bias in the estimation of  $S_{I(30)}$  by  $S_{I(12)}$  and  $S_{I(13)}$  and of  $S_{G(30)}$  by  $S_{G(12)}$  and  $S_{G(13)}$ . Non-specific alternative hypotheses were used ( $P$  values are two sided). CIs of the medians were calculated according to the nonparametric method given by Gardner and Altman (18). The IQRs of %RE data were used to gauge the precision of the reduced sampling protocol estimates. CVs calculated by MINMOD were used to assess



**FIG. 3.** Histograms of  $S_I$  and  $S_G$  %REs comparing the bias and accuracy of estimation of the reduced sampling protocols. Unbiased estimates have distributions centered around 0. Accuracy can be gauged from the spread of the distribution (the smaller the better) ( $n = 26$ ).

TABLE 2  
 $S_I$  data

	Full		Reduced					
	$S_{I(30)}$	CV (%)	$S_{I(12)}$	CV (%)	%RE	$S_{I(13)}$	CV (%)	%RE
	0.96	17	1.22	28	-27	1.25	27	-30
	0.4	54	0.42	66	-5	0.44	51	-10
	0.17	55	0.13	60	24	0.18	58	-6
	0.64	11	0.51	14	20	0.70	14	-9
	0.94	11	0.87	18	7	1.04	17	-11
	0.77	16	0.69	25	10	0.82	23	-6
	1.65	10	1.29	14	22	1.59	13	4
	1.35	10	1.18	18	13	1.37	17	-1
	0.43	24	0.32	52	26	0.39	47	9
	0.44	28	0.3	72	32	0.39	50	11
	0.67	15	0.46	28	31	0.56	25	16
	0.73	40	0.73	58	0	0.72	52	1
	0.16	117	0.28	117	-75	0.36	97	-125
	0.12	20	0.15	30	-25	0.21	29	-75
	1.72	10	1.21	13	30	1.52	14	12
	0.72	22	0.72	30	0	0.61	28	15
	0.82	18	0.69	28	16	0.7	24	15
	0.91	15	0.78	27	14	0.9	25	1
	0.12	56	0.04	236	67	0.06	243	50
	0.29	23	0.22	33	24	0.3	31	-3
	0.8	28	0.58	37	28	0.63	38	21
	1.32	18	1.40	63	-6	1.46	57	-11
	2.47	10	2.28	16	8	2.37	14	4
	1.58	11	1.46	16	8	1.75	15	-11
	0.49	22	0.32	30	35	0.46	27	6
	0.34	13	0.24	21	29	0.32	20	6
Median	0.725	18.4	0.635	29.0	15.1	0.665	27.0	1.2
IQR	0.548	15.3	0.798	37.5	25.2	0.808	31.2	20.7

$S_I$  data in  $\text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml} \times 10^4$ .

the precision of determination. Data analysis was conducted using S-Plus software (19).

**RESULTS**

Figure 1 illustrates the mean glucose and insulin concentrations from the full sampling schedule of insulin-modified FSIVGTTs (95% CIs of the means are also shown). Values calculated for  $S_I$  and  $S_G$  are given in Tables 2 and 3, respectively, together with their corresponding CVs and %REs. Median and IQR data are also given. Box-plots showing the median (white line inside the box), IQR

(height of the box), and outliers (lines outside the "whiskers") are presented for  $S_{I(30)}$ ,  $S_{I(12)}$ , and  $S_{I(13)}$  in Fig. 2A. Figure 2B depicts the corresponding information for the  $S_G$  data.

**Bias of estimates.** Table 4 gives the statistics calculated to assess the bias introduced by the reduced sampling regimens. CIs of the medians are actually 97% intervals because of the nonparametric nature of the method of calculation. Both CIs and the test statistics imply statistical support for the rejection of the null hypothesis (true median is equal to zero) for  $S_{I(12)}$ . This is not the case for

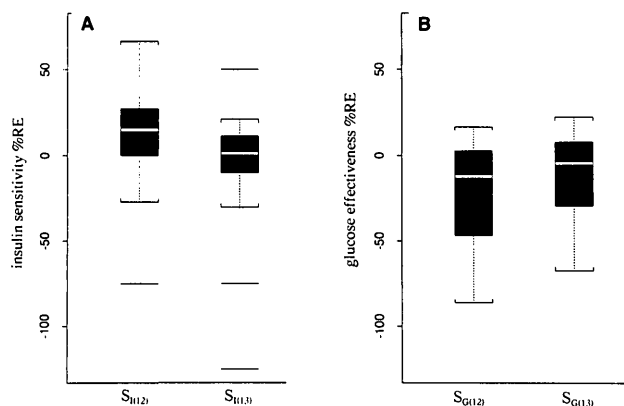


FIG. 4. A: Distributions of %REs of  $S_I$  estimates ( $n = 26$ ). B: Distributions of %REs of  $S_G$  estimates ( $n = 26$ ).

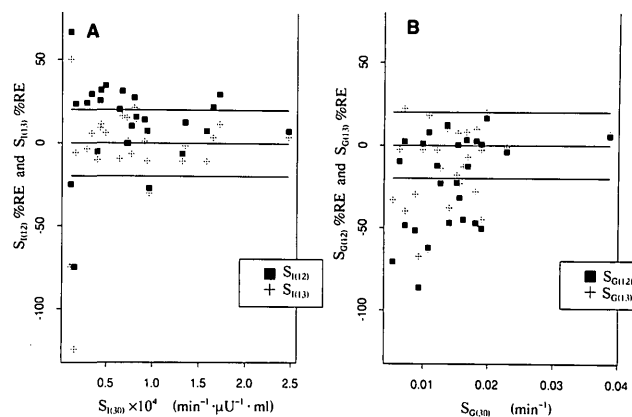


FIG. 5. A: %REs of  $S_I$  estimates vs.  $S_{I(30)}$  showing  $\pm 20\%$  RE ranges ( $n = 26$ ). B: %REs of  $S_G$  estimates vs.  $S_{G(30)}$  showing  $\pm 20\%$  RE ranges ( $n = 26$ ).

TABLE 3  
S<sub>G</sub> data

	Full		Reduced					
	S <sub>G(30)</sub> (min <sup>-1</sup> )	CV (%)	S <sub>G(12)</sub> (min <sup>-1</sup> )	CV (%)	%RE	S <sub>G(13)</sub> (min <sup>-1</sup> )	CV (%)	%RE
	0.01898	6	0.01880	9	1	0.01952	8	-3
	0.01820	6	0.01770	10	3	0.01623	10	10
	0.01668	9	0.01610	14	3	0.01528	14	8
	0.00709	15	0.00690	24	3	0.00499	33	22
	0.00630	17	0.00690	25	-9	0.00627	26	-2
	0.01537	7	0.01530	11	0	0.01416	12	8
	0.00528	22	0.00900	18	-70	0.00695	22	-33
	0.01519	6	0.01860	8	-22	0.01779	8	-18
	0.01564	9	0.02060	11	-32	0.01921	11	-23
	0.01810	8	0.02660	9	-47	0.02311	9	-28
	0.01270	6	0.01560	8	-23	0.01454	8	-14
	0.02285	7	0.02369	10	-4	0.02308	10	-1
	0.03874	3	0.03650	5	6	0.03599	4	7
	0.01369	8	0.01200	15	12	0.01215	14	10
	0.00721	16	0.01070	16	-48	0.00995	16	-40
	0.00992	13	0.00980	21	1	0.01019	19	-2
	0.01088	12	0.01000	23	8	0.00910	24	18
	0.01404	9	0.02060	9	-47	0.01935	9	-38
	0.01903	6	0.02860	7	-50	0.02810	7	-45
	0.00878	14	0.01330	16	-52	0.01098	19	-30
	0.01972	12	0.01600	23	17	0.01533	23	20
	0.01080	18	0.01750	45	-62	0.01759	41	-63
	0.00935	7	0.01740	8	-86	0.01560	8	-67
	0.01690	6	0.01900	8	-12	0.01813	8	-7
	0.01623	11	0.02350	11	-45	0.01818	14	-13
	0.01214	7	0.01360	9	-12	0.01248	9	-3
Median	0.01462	8.6	0.01675	11.1	-12.2	0.01547	11.3	-4.8
IQR	0.00766	6.4	0.00767	9.0	49.2	0.00768	10.9	36.5

S<sub>I(13)</sub>. The bias introduced by the 12-point sampling regimen was of the order of 15% ( $P = 0.0068$ ) (IQR 25%), whereas the corresponding value for S<sub>I(13)</sub> was 1% ( $P = 0.88$ ) (IQR 21%). Results for S<sub>G</sub> were equivocal. Wilcoxon's signed rank test strongly supported the rejection of the null hypothesis for S<sub>G(12)</sub> ( $P = 0.0048$ ); however, the CI includes 0. This is unusual but not impossible and may be caused by a combination of the effects of a possible bimodality in the S<sub>G(12)</sub> %RE data (Fig. 3) and the nonparametric method of calculating the CIs. The median %RE was -12% (IQR 49%) for S<sub>G(12)</sub>. Wilcoxon's signed rank test supported the rejection of the null hypothesis for S<sub>G(13)</sub> at the 5% level but not at the 1% level ( $P = 0.035$ ). The median %RE for S<sub>G(13)</sub> was -5% (IQR 37%). The histograms of Fig. 3 illustrate the shift away from 0 %RE produced by the 12 time-point regimen compared with the 13 time-point regimen.

**Precision of estimation.** Figure 3 also highlights the improved precision of the 13 time-point regimen values, increased numbers of %REs near 0 indicating closer agreement. Figures 4A and B show boxplots of S<sub>I</sub> %REs and S<sub>G</sub> %REs, respectively (S<sub>I(13)</sub> IQR 21% vs. S<sub>I(12)</sub> IQR 25%) (S<sub>G(13)</sub> IQR 37% vs. S<sub>G(12)</sub> IQR 49%). These, together with Figs. 5A and B (%REs vs. full sampling schedule estimates), demonstrate the reduction in both the size and spread of %REs obtained by introducing the 25-min time-point data. In Fig. 5, note that the outlying %RE values occur only for the lowest values of S<sub>I</sub>.

**Precision of determination.** CVs of the minimal model estimates were of the order of 18 (IQR 15%), 29 (IQR 38%), and 27% (IQR 31%) for S<sub>I(30)</sub>, S<sub>I(12)</sub>, and S<sub>I(13)</sub> and of 9 (IQR 6%), 11 (IQR 9%), and 11% (IQR 11%) for S<sub>G(30)</sub>, S<sub>G(12)</sub>, and S<sub>G(13)</sub>, respectively. Median increases in CV for S<sub>I(12)</sub> and S<sub>I(13)</sub> compared with S<sub>I(30)</sub> were 9 and

TABLE 4  
Assessment of bias estimates

	Median %RE	95% CI of median*	Z statistic†	P value
S <sub>I(12)</sub>	15.1	7.4, 25.6	2.706	0.0068
S <sub>I(13)</sub>	1.2	-9.4, 9.3	0.1524	0.8789
S <sub>G(12)</sub>	-12.2	-46.7, 1.2	-2.819	0.0048
S <sub>G(13)</sub>	-4.8	-27.8, 6.8	-2.108	0.0350

Null hypothesis: true mean %RE is equal to zero.

\*Actually 97% CIs as a result of the nonparametric method of calculation.

†Normal statistic with tie correction for Wilcoxon's signed rank test.

7%, respectively. Median increases in CV for  $S_{G(12)}$  and  $S_{G(13)}$  compared with  $S_{G(30)}$  were both 2%.

## DISCUSSION

A variety of methods exists for the estimation of  $S_1$  from plasma concentrations of glucose and insulin that include the euglycemic-hyperinsulinemic clamp (7), the FSIVGTT (1), HOMA (20), CIGMA (21), and the SITT (22). The choice of method to be used is based on the type of study and practical and logistical considerations. However, the relative ease of carrying out the FSIVGTT, HOMA, CIGMA, and SITT techniques compared with the euglycemic clamp remains attractive to investigators.

The advantages of the full sampling schedule FSIVGTT include the comparative simplicity of the technique and its ability to provide estimates of both  $S_1$  and  $S_G$ , but in its original form, the full sampling schedule requires a large number of blood samples. Based on the study of a small number of normal subjects ( $n = 10$ ), Steil et al. (13) attempted to demonstrate that a reduced sampling regimen in a tolbutamide-modified IVGTT could adequately estimate  $S_1$  and  $S_G$  in a wider group ( $n = 87$ , with a total of 118 tolbutamide-modified FSIVGTTs), including healthy subjects, the aged, and subjects with gestational diabetes or drug-induced insulin resistance, and as such would be suitable for use in certain population studies. Our study addresses the specific questions about the introduction of bias and the precision of estimation and determination of  $S_1$  and  $S_G$  from two reduced sampling regimens used during an insulin-modified IVGTT in NIDDM subjects treated with diet only. In method-comparison studies, these questions cannot be adequately answered by use of correlation and regression analyses (18,23).

In this study, statistically significant and clinically important bias was introduced by the use of reduced sampling regimens in the case of  $S_{1(12)}$  but not  $S_{1(13)}$ . Results for  $S_{G(12)}$  and  $S_{G(13)}$  were statistically equivocal, but the introduction of bias cannot be discounted. Introduction of the 25-min time-point data resulted in improvement in the accuracy and precision of estimation of the  $S_{1(13)}$  values. The precision of estimation of the  $S_{1(12)}$ ,  $S_{G(12)}$ , and  $S_{G(13)}$  values was substantially decreased. This time point was introduced empirically because of concerns over the reconstruction of the insulin profile in the critical period after the insulin bolus at 20 min. The precision of determination of  $S_1$  was markedly reduced for both  $S_{1(12)}$  and  $S_{1(13)}$ , but this was not the case for  $S_{G(12)}$  and  $S_{G(13)}$ . The CVs reported here appear larger than those reported originally by Bergman (1) and recently by Steil et al. (13), but this may be a result of the low  $S_1$  values estimated in these NIDDM subjects. When taken in combination, these effects may accumulate to such an extent that the resulting values are of little use even for large population studies. Power calculations to determine the actual size of such studies have yet to be made.

In a wider context, since the introduction of minimal modeling of the FSIVGTT, the methodologies used by investigators have varied considerably. Unmodified

FSIVGTTs have been used to investigate subjects in whom the insulin response is adequate for modeling (5,6,24,25). Even within these studies, however, the sampling regimens have varied from 10 (6) to 19 time points (24). Additionally, a recent study in individuals at risk of NIDDM in whom the insulin response cannot be presumed to be unimpaired used an unmodified IVGTT with a sampling regimen of 13 time points (26). A modification of the FSIVGTT has been introduced to augment the insulin response in normal subjects using a single intravenous dose of tolbutamide (300 mg) regardless of subject size (3) or varying the dose according to BSA (8,13). An insulin-modified FSIVGTT protocol in subjects with NIDDM has used insulin either as an intravenous bolus (12) or an infusion over 5 min (27). The MINMOD program also exists in several versions with possible additional modifications having been made by the individual investigators without adequate documentation. Minimal modeling analysis of the unmodified and modified FSIVGTT remains a useful and flexible technique for the investigation of  $S_1$ , but it clearly needs standardization with guidelines for the appropriateness of protocol modifications depending on the questions to be answered by the investigation and the subjects to be investigated.

In NIDDM, we conclude that the 12 time-point protocol for the IVGTT in conjunction with MINMOD analysis should be restricted to large population studies. A 13 time-point protocol as described here is clearly more acceptable for the estimation of  $S_1$  in large population studies of NIDDM but still cannot be recommended for the assessment of  $S_G$ , a fact appreciated and commented on by Steil et al. (13). Therefore, for clinical research in subjects with NIDDM, we regard the retention of the full sampling protocol to be prudent.

## ACKNOWLEDGMENTS

We are grateful to Sister Hilary Smith and nurse Miriam Khan for conducting the FSIVGTTs, Peter Ashley for the assays, and Katherine Srodzinski for data preparation.

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