Derivation of a quantitative measure of insulin sensitivity from the intravenous tolbutamide test using the minimal model of glucose dynamics

N. M. Shennan, I. F. Godsland and V. Wynn

The Cavendish Clinic, London, UK

Summary. Using the decay phase of the glucose response during an intravenous tolbutamide test, a minimal model of glucose dynamics was used to calculate a value for an “index of insulin sensitivity”. This index describes the efficiency of insulin in accelerating the instantaneous rate of glucose disposal, and provides a measure of insulin resistance. The validity of estimates of the index of insulin sensitivity obtained from the intravenous tolbutamide test have been assessed with reference to estimates of this index derived from the intravenous glucose tolerance test for which the model was originally designed. There were three studies: (A) estimates of the index of insulin sensitivity obtained from the intravenous tolbutamide test in a group of normal, healthy men and women were compared with results obtained in a comparable group of subjects using the intravenous glucose tolerance test. The two methods gave estimates of the index of insulin sensitivity that were identical; (B) A group of patients taking methandienone, an anabolic steroid previously shown to cause marked insulin resistance, were tested whilst taking the steroid and either before, or at least two months after treatment. Each patient was tested by both intravenous tolbutamide test and intravenous glucose tolerance test on both occasions. Estimates of the index of insulin sensitivity from intravenous glucose tolerance or intravenous tolbutamide procedures both on and off treatment were significantly correlated (off treatment: $r_s=0.71$, $n=9$, $p<0.05$; on treat-

ment: $r_s=0.69$, $n=9$, $p<0.05$); (C) A group of patients undergoing investigations for suspected disturbances in carbohydrate metabolism was studied, each patient having had both an intravenous tolbutamide and intravenous glucose tolerance test. The group studied included patients in whom a degree of insulin resistance would be expected. Estimates of the index of insulin sensitivity from the two methods were closely correlated ($r_s=0.95$, $n=25$, $p<0.001$). This strong, identical correlation obtained between the intravenous glucose tolerance and intravenous tolbutamide tolerance estimates of index of insulin sensitivity in studies B and C over a wide range of values [intravenous tolbutamide tolerance test: $0.11\text{--}1.07\text{ min}^{-1}\text{U}^{-1}$ l; intravenous glucose tolerance test: $0.12\text{--}1.06\text{ min}^{-1}\text{U}^{-1}$ l]. This suggests that the intravenous tolbutamide estimates of index of insulin sensitivity are closely comparable to those derived from intravenous glucose tolerance test over a broad range of insulin sensitivities. We suggest that the use of intravenous tolbutamide to induce a dynamic change in insulin-glucose relationships, and mathematical modelling of those dynamics, can provide a valuable, quantitative measure of insulin sensitivity in a variety of clinical situations.

Key words: Insulin sensitivity, intravenous tolbutamide test, intravenous glucose tolerance test, insulin action, glucose dynamics, mathematical modelling.

The minimal model of glucose dynamics has been used to derive estimates of an index of insulin sensitivity (S_I) from the intravenous glucose tolerance test (IVGTT) in man [1]. S_I is defined mathematically as a ratio of two rate constants governing (a) the action of insulin via the plasma from a remote compartment, I , on the sites of active glucose disposal and (b) the egress of insulin from this remote compartment (and hence the diminution of its action). Such a quantity is potentially useful to clinicians and research workers, as it offers a quantitative measure of the effectiveness of insulin in accelerating glucose disposal.

Although the minimal model was originally designed for use where plasma glucose concentrations are elevated, it is theoretically used whenever plasma glucose concentrations are falling because of a rise in insulin secretion. During an intravenous tolbutamide test (IVTT), where sulphonylurea stimulation of the islet B cell increases insulin secretion, the plasma glucose concentration falls from the basal level to a nadir. During a fall in plasma glucose concentrations following such an increase in insulin secretion, it has been demonstrated [2–6] that the plasma concentrations of the principal counter-regulatory hormones (glucagon,

cortisol, catecholamines and growth hormone), barely respond for approximately 30 min, at which point the plasma glucose is still roughly 20% above its eventual nadir. Furthermore, it has been demonstrated that glucose production drops continuously over this period [2] consistent with the inhibitory effect of elevated plasma insulin concentrations. Thus, with glucose disposal unopposed, a continuous rise in net glucose utilisation is observed in response to a sustained elevation in plasma insulin concentrations. It would appear, therefore, that the action of insulin on net glucose disposal within the first 30 min following the administration of tolbutamide is unconfounded by the effects of other counter-regulatory glucostatic hormones. Consequently, application of the minimal model during this period to derive estimates of S_I is valid.

In the present study, we have compared estimates of S_I derived from the modelling of plasma glucose concentrations obtained during both IVTTs and IVGTTs in three groups of subjects in which a wide range of insulin sensitivities may be expected. We have excluded cases of obesity or diabetes mellitus from the present study. However, we are currently studying these other conditions using the techniques described.

Subjects and methods

Subjects

The three groups of subjects studied were as follows:

Study A. Normal healthy non-obese men and women were selected following a thorough medical examination, including reference to exhaustive lists of drugs and diagnoses associated with disturbances of carbohydrate metabolism. Fifty-eight cases were studied, 30 having IVGTTs (15 male, 15 female) and 28 having IVTTs (13 male, 15 female). There were no significant differences in age and body mass index (BMI) between the IVGTT study group (age range: 19–58 years; BMI range: 19.4–23.0 kg/m²) and IVTT study group (age range: 20–51 years; BMI range: 18.5–24.3 kg/m²).

Study B. Nine patients (2 men, 7 women) were studied whilst on treatment and either before or after treatment with the anabolic steroid, methandienone. The indication for this treatment was severe weight loss following a period of negative nitrogen balance associated with post-operative recovery or anorexia. The patients had both an IVGTT and an IVTT. In 7 cases tests were carried out prior to commencing treatment and then on treatment. The remainder were tested on treatment, and then when off treatment for at least 2 months. Dosages of methandienone were 10 mg (4 subjects), 15 mg (1 subject) or 20 mg (4 subjects) per day. This group has been discussed in detail elsewhere with respect to the mathematical modelling of IVGTT data [8]. In the current analysis, the group has been examined in order to compare estimates of S_I derived from both IVTT and IVGTT procedures. These experiments were carried out around 1960, at a time when ethical committee approval had not yet been institutionalised in the UK.

Study C. Twenty-five patients undergoing investigation for suspected disturbances in carbohydrate metabolism were included in this study. Patients had both an IVGTT and an IVTT. Two men proved to be healthy, 5 men and 2 women had previously shown evidence of reactive hypoglycaemia, 3 men had glycosuria, 1 man was acromegalic and 2 women had Cushing's syndrome. The group also in-

cluded seven women taking a variety of oral contraceptives, and 1 woman and 2 female transsexuals receiving testosterone enanthate.

All volunteers and patients had the procedures explained to them and gave their informed consent. Patients in study B were being investigated for the metabolic effects of methandienone and related compounds. The clinical decision to administer methandienone was the responsibility of one of the authors (VW). Patients in study C were undergoing detailed investigations into pancreatic function and glucose dissimulation in relation to diagnosis and monitoring of disorders of carbohydrate metabolism. Use of the procedures described in this study has the approval of the Paddington and North Kensington Area Health Authority Ethical Committee.

Methods

Prior to testing, all subjects had followed a diet containing approximately 300 g of carbohydrate per day for 3 days and had fasted from 21.00 hours the previous evening. Tests commenced at 09.00 hours and patients remained semi-recumbent throughout each test. Cannulae were introduced into an antecubital vein in both arms under local anaesthetic, and after 20 min bed rest two blood samples were withdrawn 10 min apart from one cannula for the determination of basal glucose and insulin concentrations. The mean concentrations for these samples were taken as the basal values. Throughout both procedures, heparin in saline was used to ensure patency of the sampling-site cannula. Samples were collected into tubes containing lithium heparin as an anti-coagulant, and were immediately centrifuged. The plasma was separated, and aliquots for the determination of glucose concentrations were stored at 4°C and assayed on the same day. Those for plasma insulin determination were stored at –20°C and assayed within 1 month.

IVGTT procedure. Glucose was injected into the cannula contralateral to that from which the baseline samples were taken. Dextrose (50% in water) was injected at 0.5 g per kg body weight over 2 min, the cannula position having been checked to ensure that extravasation did not occur. Samples (10 ml) of venous blood were withdrawn from the sampling site on the contralateral arm. They were taken from the start of glucose injection: 1, 2, 3, 5, 7, 10, 20, 30, 45, 60, 75, 90 min.

IVTT procedure. An injection of tolbutamide (1 g) in saline was given over 1 min into the cannula contralateral to that from which the baseline samples were taken. Samples (10 ml) of venous blood were withdrawn from the contralateral arm from the start of tolbutamide injection: 1, 2, 3, 5, 7, 10, 20, 30, 45, 60, 75, 90, 105, 120 min.

Biochemical analyses. Plasma glucose concentrations were measured on a Technicon Autoanalyser using the glucose oxidase method of Cramp [10]. Within and between assay coefficients of variation (CVs) were 1.6% and 1.6%, respectively, at a mean glucose concentration of 8 mmol/L. Plasma insulin levels were measured in duplicate by the specific radioimmunoassay of Albano et al. [11], with single incubation and charcoal separation, and reagent conditions selected to give maximum sensitivity over the range of elevated insulin concentrations likely to be encountered during an IVGTT or IVTT. The within and between assay CVs for this assay were 6.6% and 9.8%, respectively. In addition, fasting insulin concentrations were determined by a disequilibrium modification of the insulin method, with the dilution of reagents increased to give a tenfold increase in sensitivity.

Modelling analysis. The minimal model of glucose dynamics has been described in detail elsewhere by the originators of the technique [12, 13], and by ourselves in a previous study of the metabolic effects of the anabolic steroid, methandienone [8].

Briefly, the minimal model of glucose dynamics employs two first-order non-linear differential equations, one describing the influence of insulin, acting from a compartment, I , remote from the plasma, on the disappearance of glucose from the plasma, and another which predicts the change in the moment-to-moment action of insu-

lin, based on its input to the sites of glucose disposal, and on its own degradation.

In its application to the IVGTT, during which there is marked hyperglycaemia, the minimal model has to take into account both insulin-dependent and insulin-independent glucose disposal. Two parameters are thus derived which quantify these different aspects of glucose disposal, namely the insulin-dependent parameter, S_I and the insulin-independent parameter, S_G .

To derive the differential equations that describe the phase of insulin-induced glucose concentration decay following stimulation of insulin secretion by intravenous tolbutamide, we begin with the basic model originally established to describe glucose dynamics following intravenous injection of glucose.

$$\dot{G} = -(k_1 + k_5) \cdot G_t - X_t \cdot G_t + p_4 \quad (1)$$

where:

$\dot{G}$ equals the change in plasma glucose concentration per unit time; k_1 equals the rate constant governing insulin-independent disposal of glucose into the peripheral tissues, k_5 the rate constant governing the insulin-independent net hepatic glucose balance, G_t the plasma glucose concentration at time, t ; X_t the net action of insulin exerted from the remote compartment on the sites of glucose disposal, and p_4 the extrapolated net glucose balance at zero time. Algebraically, this is equivalent to the expression $(k_1 + k_5) \cdot G_b$, where G_b is the basal glucose concentration.

The results of studies by Rizza et al. [14], and by Bergman and Bucolo [15] suggest that, at plasma insulin concentrations normally experienced during the phase of decline in plasma glucose concentrations in an IVTT, hepatic glucose production would be rapidly and completely suppressed. Thus, it is assumed that the term p_4 does not contribute significantly to the plasma glucose dynamics during this time. In addition, results from studies by Best et al. [16] can be used to calculate that, during the same period of an IVTT, 95% of the change in net glucose disposal rate is accounted for by insulin-dependent glucose uptake. It is thus reasonable to assume that changes in insulin-independent glucose disposal make a negligible contribution to glucose dynamics during this phase. Thus, we arrive at the following equation:

$$\dot{G} = -X_t \cdot G_t \quad (2)$$

Using the same notation of Eq. (1) above, the instantaneous change in insulin action is given by another first-order non-linear differential equation:

$$\dot{X} = -k_3 \cdot X_t + k_2 \cdot (k_4 + k_6) \cdot I_t \quad (3)$$

where:

$\dot{X}$ equal change in insulin action from the remote compartment per unit time; k_2 the rate constant governing the ingress of insulin to the compartment, I_t remote from the plasma; k_3 the rate constant describing the declining action of insulin from the remote compartment; k_4 the rate constant describing the remote action of insulin on peripheral glucose disposal; k_6 the rate constant describing the remote action of insulin on the hepatic glucose balance; I_t the plasma insulin concentration at time, t ; X_t the net remote insulin action at time, t .

Modelling of IVTT glucose concentrations on the plasma insulin concentrations may then provide solutions of Eqs. (2) and (3) in terms of values for the combined rate constants $[k_2 \cdot (k_4 + k_6)]$ which govern insulin-mediated glucose disposal and k_3 which governs the declining action of insulin in the remote compartment.

The sensitivity of the mechanism of glucose disposal to insulin can then be expressed as a ratio of these rate constants:

$$S_I = [k_2 \cdot (k_4 + k_6)] / k_3$$

S_I will then have units of $\text{min}^{-1} \text{U}^{-1} \text{I}$.

Computational methods. All analyses were performed on a DEC PDP-11/23 minicomputer running on an RSX-11M operating system. Reiterative solution of the differential equations by nonlinear least-squares estimation was performed using a program written in FORTRAN-77 and PDP-11 MACRO Assembler and based on the algorithm of Marquardt [17]. Fibonacci [18], and gradient [19] searches were used to optimise the interim parameter estimates until the minimal value of the residual sums of squares was found. Revised parameter estimates between iterations were derived by expansion of the increment given by:

$$\Delta p = [S^T R^{-1} S]^{-1} S^T R^{-1} \Delta z \quad [20]$$

where:

S is the sensitivity matrix, composed of the gradient equations in terms of each parameter to be estimated; R is the matrix of measurement errors; Δz is the vector of differences between observed and model-derived glucose values at each time point, for the specific parameter estimates of the n^{th} iteration.

The increment, Δp , was then used to update the matrix of parameter estimates prior to the next (n^{th}) iteration by:

$$p^n = p^{n-1} + \Delta p.$$

The measurement error matrix was constructed assuming a constant, mean within-batch coefficient of variation of the plasma glucose assay of 1.6%, with an extra, presumed additive, component due to sample-timing and possible cross-contamination of 1%, giving a total measurement error variation of 2.6% at each time point, t . It was also assumed that errors were non-systematic and Gaussian with a mean of 0. These criteria were also used to compute the measurement error sums of squares described below.

The program was written with a subroutine to determine the change in the residual sums of squares (SS_r) computed for the difference between the observed and the model-predicted glucose values from the Monte Carlo simulation using the final parameter estimates. This change was evaluated over every five iterations, in order to establish that point at which the SS_r did not improve successively by more than 1%, and cumulatively by more than 2.5%. In general, this was achieved within 35 iterations per individual. Final fractional standard deviation (FSD) estimates, as indications of the accuracy of the individual parameter estimates, were computed at convergence for each reiterative analysis from the principal diagonal of the Fisher information matrix, J , which was formed according to Di Stefano [18], and Carson et al. [20]. In addition the SS_r from the fitting of the simulated to the observed glucose data were compared to the error sums of squares SS_m (computed from the criteria above) for the plasma glucose measurements at the end of each individual analysis. Bergman et al. [21] have advocated the use of the index $\sqrt{[SS_r/SS_m]}$ as an indicator of the accuracy with which a given model approximates the observed data, and this has been used to compare the numerical efficiency of different models. This approach has been adopted in the current study, and the term "suitability index" has been used for brevity.

Although plasma glucose and insulin data in the IVTT were collected from 0 to 20 min, in no individual did the plasma glucose profile show any consistent decrease in the first 3 min after tolbutamide administration, even though plasma insulin concentrations were already considerably higher than the basal level. This delay corresponds to that period in which insulin distribution is taking place, and only after this has occurred will the action of insulin on plasma glucose be measurable. Consequently, only data spanning the period from 3 to 20 min were used. To accomplish this, the algorithm incorporated zero-weighting, derived from a Kronecker delta sequence [22], such that:

$$w(t) = 1 \text{ if } 3 \leq t \leq 20 \text{ min} \\ w(t) = 0 \text{ otherwise.}$$

All other statistical analyses were performed using the Statistical Package for the Social Sciences, adapted for the PDP-11 [23].

Table 1. Medians of age (years), BMI (kg/m^2), S_I ($\text{min}^{-1}\text{U}^{-1}$), fractional standard deviation (FSD, %) of S sums of squares of measurement errors (SS_m), and of residuals (SS_r), and the suitability index ($\sqrt{[\text{SS}_r/\text{SS}_m]}$) of Study A. The suitability index is a measure of model goodness-of-fit. Actual ranges are given throughout

Sex	Test	Age	BMI	S_I	FSD (%)	n	SS_m	SS_r	Suitability index
Males	IVGTT	26	22.5	0.92	5.3	15	3.29	6.52	1.42
Range:		21–58	20.9–23.0	0.86–0.99	3.3–6.0		2.23–3.85	4.92–7.18	1.13–1.78
Males	IVTT	26	22.0	0.88	5.3	13	1.64	3.52	1.45
Range:		22–51	21.3–24.3	0.82–0.99	3.1–6.0		1.02–2.07	1.97–4.29	1.21–1.73
Females	IVGTT	23	20.7	0.92	5.1	15	2.99	5.87	1.37
Range:		19–47	19.4–21.7	0.89–1.09	3.0–6.0		1.88–3.85	4.60–6.79	1.09–1.58
Females	IVTT	24	20.2	0.90	4.9	15	1.27	2.57	1.39
Range:		20–49	18.5–23.5	0.88–1.01	3.0–5.3		0.98–1.70	1.71–3.63	1.11–1.65

Fig. 1. Plasma insulin (upper) and glucose (lower) concentration profiles during a 120-min intravenous tolbutamide test (IVTT) in nine patients on (---▲---) or off (—△—) treatment with methandienone; data are shown as mean $\pm$ SE

Statistical analysis

The agreement between the estimates of S_I derived from the IVGTT and IVTT procedures was tested using the Spearman rank correlation coefficient [24]. Deviations from a line of equality (i.e. along which S_I (IVGTT) = S_I (IVTT)) was quantified using a standardised residual mean square for each pair of values of S_I computed. The deviation of the estimate made by the IVTT procedure was expressed as a residual (d_{yx}) of the line of equality, and plotted against the estimate made by the IVGTT procedure. Therefore:

$$d_{yx} = S_I [\text{IVTT}] - S_I [\text{IVGTT}].$$

Expressing the line as a linear equation in x of the general form:

$$y = bx + a$$

the error residual mean square becomes:

$$S_{yx} = [(y_i - a - bx_i)]^2 / \text{degrees of freedom (n of pairs)} \\ = [(y_i^2 (a + bx_i)y_i + (a + bx_i)^2)/n.$$

Table 2. Individual estimates of S_I derived from the two procedures in Study B. Analyses listed according to status of treatment with methandienone. Legend otherwise as Table 1

Subject	Status	IVGTT					IVTT				
		S_I	FSD (%)	SS_r	SS_m	$\sqrt{[SS_r/SS_m]}$	S_I	FSD (%)	SS_r	SS_m	$\sqrt{[SS_r/SS_m]}$
1	Before	0.69	3.8	6.42	3.38	1.38	0.60	5.0	3.63	1.82	1.41
1	During	0.12	4.4	6.13	2.86	1.46	0.15	5.1	2.95	1.61	1.36
2	Before	0.76	3.8	5.69	2.89	1.41	0.70	4.4	2.98	1.53	1.41
2	During	0.15	5.5	5.94	3.75	1.26	0.13	4.4	3.22	1.53	1.41
3	Before	0.71	5.5	6.90	3.70	1.37	0.80	4.5	4.05	2.00	1.42
3	During	0.17	4.0	6.69	3.06	1.48	0.11	5.2	2.97	1.52	1.40
4	Before	0.81	4.5	5.08	2.49	1.43	0.95	5.1	3.16	1.51	1.44
4	During	0.21	2.3	5.64	2.88	1.40	0.17	3.4	2.95	1.64	1.34
5	Before	0.83	3.9	7.06	3.81	1.36	0.98	3.0	4.19	1.76	1.54
5	During	0.21	5.5	6.09	3.07	1.41	0.20	4.5	4.15	1.91	1.47
6	Before	0.82	5.0	6.27	3.28	1.38	0.98	5.1	3.71	1.89	1.40
6	During	0.22	5.7	5.65	2.64	1.46	0.25	4.8	3.49	1.71	1.43
7	After	0.92	3.4	6.61	3.51	1.37	0.87	4.4	3.80	2.03	1.37
7	During	0.20	5.0	5.30	3.02	1.32	0.30	4.3	2.11	1.92	1.42
8	After	1.01	3.0	4.43	2.07	1.46	1.06	3.4	1.91	1.05	1.35
8	During	0.29	4.9	4.26	2.07	1.43	0.39	4.4	2.66	1.39	1.39
9	After	1.06	4.6	4.36	2.02	1.47	1.07	5.0	1.85	1.02	1.34
9	During	0.31	4.4	5.88	3.19	1.36	0.27	5.6	1.78	0.98	1.34

S_I medians and ranges:

	IVGTT	IVTT
Before and after:	0.82 [0.69–1.06]	0.95 [0.60–1.07]
During:	0.21 [0.12–0.31]	0.20 [0.11–0.39]

In this case, where $a=0$ and $b=1$, the expression reduces to:

$$S_{yx} = [(y_i - x_i)^2 / n] \\ = [d_{yx}^2 / n].$$

The individual deviations were expressed as a percentage of the residual mean square and analysed using the Spearman rank correlation coefficient to test if the difference in the estimates from the two methods was associated with the magnitude of the original IVGTT-derived estimate. The individual values of the differences, d_{yx} , were tested for deviation from a Gaussian distribution with a Kolmogorov-Smirnov goodness-of-fit test [23, 24], and the proximity of the differences to a mean value of zero was estimated using a Wilcoxon paired-signed ranks test [23, 24].

Mann-Whitney U-tests and Wilcoxon paired signed-rank tests were used for all between-, and within-group parameter comparisons [24].

Results

Study A

Median age, BMI, S_I , FSD of the S_I estimate, residual and measurement error sums of squares, and the suitability index for control subjects in Study A are summarised in Table 1. For the sexes combined, the total range of estimates of S_I was contained within narrow bounds for both tests (IVGTT: 0.86 – $1.09 \text{ min}^{-1} \text{U}^{-1} \text{l}$; IVTT: 0.82 – $1.01 \text{ min}^{-1} \text{U}^{-1} \text{l}$), and the median values of S_I also showed good agreement (IVGTT: $0.92 \text{ min}^{-1} \text{U}^{-1} \text{l}$; IVTT: $0.89 \text{ min}^{-1} \text{U}^{-1} \text{l}$; NS). The FSDs of the estimates ranged from 3 to 6%. The suitability index showed no significant difference between the modelling analyses applied to the two methods and

Fig. 2. Lower plots: Insulin sensitivity index, S_I , estimated from the results of (abscissa) intravenous glucose tolerance tests (IVGTTs) scattered against (ordinate) S_I index estimates from intravenous tolbutamide tests (IVTTs) for patients off ($\square$) or on ($\blacksquare$) treatment. Correlations were $r_s=0.71$ ($p<0.05$) off treatment and 0.69 ($p<0.05$) on treatment ($n=9$). Upper plots: Percentage deviations from the scatter plot described above, compared to the line of equality. The percentage deviation is the quantity $100 [d_{yx}/SR_{yx}]$ where d_{yx} is the individual absolute deviation for a given point, and SR_{yx} is the residual sums of squares of the points from the line $y=bx$. The percentage deviation for the IVTT estimates of S_I have been plotted against the corresponding values of S_I determined from the IVGTT procedure; $r_s=0.36$ ($n=9$, NS).

Table 3. Individual estimates of S_I derived from the two procedures in Group C (miscellaneous subjects). Legend as in previous tables

Subject	Diagnosis	IVGTT					IVTT				
		S_I	FSD (%)	SS_r	SS_m	$\sqrt{[SS_r/SS_m]}$	S_I	FSD (%)	SS_r	SS_m	$\sqrt{[SS_r/SS_m]}$
1	Acromegaly	0.14	4.6	6.99	4.54	1.24	0.20	4.0	3.76	1.86	1.42
2	Cushing's syndrome	0.19	4.0	5.75	3.02	1.38	0.28	5.3	2.90	1.49	1.40
3	Cushing's syndrome	0.27	2.9	4.25	1.92	1.49	0.26	3.1	1.71	0.96	1.33
4	Hypoglycaemia	0.38	4.2	5.28	2.57	1.43	0.49	2.3	3.29	1.66	1.41
5	Hypoglycaemia	0.46	3.4	4.65	2.69	1.31	0.56	3.0	1.74	1.24	1.19
6	Hypoglycaemia	0.49	4.6	5.79	3.29	1.32	0.41	5.0	2.98	1.49	1.41
7	Hypoglycaemia	0.51	5.1	5.57	3.09	1.34	0.56	4.9	3.78	1.87	1.42
8	Hypoglycaemia	0.53	3.0	4.34	1.94	1.50	0.52	3.0	1.71	0.98	1.32
9	Hypoglycaemia	0.57	4.3	5.80	3.94	1.21	0.60	4.2	2.93	1.52	1.39
10	Hypoglycaemia	0.77	4.0	5.18	2.62	1.41	0.79	3.7	2.66	1.38	1.39
11	Glycosuria	0.61	3.0	4.94	2.82	1.32	0.60	5.0	2.23	1.62	1.17
12	Glycosuria	0.90	3.2	4.49	2.15	1.45	0.96	3.0	2.02	1.19	1.30
13	Glycosuria	0.92	5.4	6.44	3.15	1.43	1.01	3.4	2.74	1.41	1.39
14	Testosterone (female)	0.27	3.0	4.11	1.91	1.47	0.34	2.8	2.72	1.35	1.42
15	Testosterone (transsexual)	0.49	4.0	4.23	2.09	1.42	0.57	4.6	2.53	1.17	1.47
16	Testosterone (transsexual)	0.56	5.5	7.21	3.75	1.39	0.58	5.8	4.19	2.05	1.43
17	OC	0.56	6.6	6.61	3.45	1.38	0.75	4.4	3.73	1.85	1.42
18	OC	0.67	3.4	4.61	1.51	1.75	0.62	4.0	2.18	1.10	1.41
19	OC	0.73	4.4	6.49	3.29	1.40	0.70	5.0	3.38	1.25	1.65
20	OC	0.77	4.6	6.51	3.54	1.36	0.76	5.0	3.92	2.48	1.26
21	OC	0.80	4.5	5.12	2.66	1.39	0.77	5.0	2.59	1.35	1.39
22	OC	0.77	4.5	6.67	3.40	1.40	0.89	5.1	3.58	1.78	1.42
23	OC	0.81	5.1	6.45	3.04	1.46	0.81	5.0	3.23	1.63	1.41
24	Normal male	0.93 ^a	4.0	4.99	2.52	1.41	0.95	4.1	2.76	1.40	1.41
25	Normal male	0.93 ^a	4.0	4.92	2.56	1.38	0.96	5.5	2.60	1.43	1.35
Median:		0.57					0.62				
Range:		[0.14–0.93]					[0.20–1.01]				

OC – oral contraceptive steroids. ^a No recorded abnormalities or drugs

the ranges of estimates of this measure, for the whole study, were very consistent between the two methods (IVGTT: 1.09–1.78, overall median 1.38; IVTT: 1.11–1.73, overall median 1.42).

Study B

The IVGTT plasma glucose and insulin concentration profiles for the subjects of Study B have been described elsewhere and show marked impairment of glucose tolerance, associated with an elevated insulin response [8, 9]. Mean IVTT plasma glucose and insulin concentration profiles for these subjects are given in Figure 1. The IVTT insulin response was markedly increased by treatment with methandienone ($p < 0.001$). A three-fold increase in both the peak insulin concentration and the area under the IVTT insulin profile was observed, although the time at which the insulin peak was reached was unchanged. The fasting plasma glucose levels were significantly reduced on treatment ($p < 0.01$), as previously observed. Although both the appearance of the nadir in the plasma glucose concentration and the recovery from hypoglycaemia were delayed ($p < 0.01$), the plasma glucose concentration at the nadir was unchanged. These results suggest that methandienone treatment causes insulin resistance.

Estimates of S_I for the subjects of Study B are given in Table 2. Values of S_I of the off treatment tests were comparable with the values for the Study A controls. However, treatment with methandienone resulted in a four-fold reduction in the index of insulin sensitivity, S_I , the same result that was obtained using the IVGTT. Individual IVTT data, and the residual differences between estimates from the two methods are plotted relative to the IVGTT-derived values of S_I in Figure 2. The correlation between S_I values derived from the two procedures was significant for both the on-, and off-treatment tests (off treatment $r_s = 0.71$, $n = 9$, $p < 0.05$; on treatment $r_s = 0.69$, $n = 9$, $p < 0.05$). No significant trend was found in the residual errors for either the off treatment or the on treatment analyses, and there was no evidence of a significant deviation from a zero mean value in these errors. The paired suitability indices for neither the off treatment nor the on treatment analyses showed any significant difference between the IVGTT and IVTT models. These observations suggest that the errors between the IVTT estimates of S_I and those from the IVGTT were not influenced by systematic error, and were distributed normally along the line of equality. It is also evident from the analysis of the suitability indices that, as in the previous study, the numerical efficiencies of the

Fig. 3. Lower plots: insulin sensitivity index, S_I , estimated from paired IVTTs and IVGTTs in patients with ($\diamond$) acromegaly, ($\blacklozenge$) Cushing's syndrome, ($\triangle$) hypoglycaemia, and ($\blacktriangle$) glycosuria, in ($\circ$, $\bullet$) transsexual subjects taking testosterone, in ($\square$) women taking oral contraceptives, and in ($\blacksquare$) two normal men; $r_s = 0.95$, ($n = 25$, $p < 0.001$). Upper plots: scatter plot of percentage deviations from the line of equality as described in the legend to Figure 2; $r_s = 0.21$ ($n = 25$, NS)

two models in producing acceptable Monte Carlo estimations of the observed data were not significantly different.

Study C

For the subjects in Study C, the correlation between S_I values derived from the IVGTT and IVTT procedures was strong ($r_s = 0.95$, $n = 25$, $p < 0.001$). Individual estimates of S_I are given in Table 3. The range of S_I in Study C was 0.20 – $1.01 \text{ min}^{-1} \text{U}^{-1} \text{l}$ for the IVTTs and 0.14 – $0.93 \text{ min}^{-1} \text{U}^{-1} \text{l}$ for the IVGTTs. Individual IVTT data, and the residual differences between estimates from the two methods are plotted relative to the IVGTT-derived values of S_I in Figure 3. No significant trend was found in the residual errors and no deviation from a Gaussian distribution of zero mean was detectable. The suitability indices for the two methods were not significantly different and the ranges of this index for the two methods were very similar (IVGTT: 1.21 – 1.75 , median 1.40 ; IVTT: 1.17 – 1.65 , median 1.41).

S_I for the two normal males and two of the subjects with glycosuria gave values within the range encountered in the Study A control subjects. The seven women taking oral contraceptives, the two transsexuals receiving testosterone enanthate, one patient with glycosuria, and the cases under investigation for reactive hypoglycaemia all showed reduced insulin sensitivity compared with controls. The two patients with Cush-

ing's syndrome, the patient with acromegaly and the female patient receiving testosterone enanthate showed the lowest values of S_I .

Discussion

Aspects of insulin insensitivity, particularly hyperinsulinaemia, are a common thread running through the associated conditions of obesity [25], Type 2 (non-insulin-dependent) diabetes mellitus [26], aging [27], hypertension [28], and cardiovascular disease [29, 30]. Insulin resistance also results from glucocorticoid excess, and from the administration of a variety of gonadal steroids [31] and is a feature of certain pathological conditions, such as acanthosis nigricans [32] and polycystic ovary syndrome [33]. Evaluation of the importance of insulin insensitivity in all these conditions has been limited by the nature of the techniques available for its quantification.

The reference technique for measurement of insulin sensitivity is generally accepted to be the euglycaemic clamp, according to which the rate of glucose disposal at a given concentration of insulin is determined in a steady state. However, in order to overcome the mutual feedback by which glucose and insulin concentrations are maintained, continuous infusions of insulin and glucose are required and there is sustained hyperinsulinaemia throughout the procedure. Glucose clamp techniques are thus invasive research procedures that impose a physiological equilibrium on the experimental subject that is far from normal [13].

An alternative to such steady-state techniques is mathematical modelling of dynamic changes in plasma glucose and insulin concentrations following a disturbance in either of these parameters. One of the most promising of these techniques is the application of the minimal model of glucose dynamics to predict plasma glucose concentrations from plasma insulin concentrations following intravenous injection of glucose. The parameter of insulin sensitivity thus derived is termed S_I [1]. By comparison with the euglycaemic clamp, the IVGTT is relatively easy to perform and may be considered as a diagnostic procedure. However, it requires injection of a large volume of substance, with a small but significant risk of thrombophlebitis ensuing. In the present study we have explored the application of the minimal model of glucose dynamics to changes in plasma glucose and insulin concentrations following stimulation of insulin secretion by intravenous injection of tolbutamide. According to this procedure a smaller volume of substance is injected and the resulting fall in plasma glucose concentrations is transient and rarely sufficient to cause symptoms of hypoglycaemia. Furthermore, it was of interest to explore the comparability of values of S_I derived conditions of both hypo-, and hyperglycaemia.

It is apparent from the results presented above that estimates of S_I derived from the IVGTT and IVTT are virtually identical and that this identity holds over a wide range of values of S_I [0.11 – $1.07 \text{ min}^{-1}\text{U}^{-1}$]. The assumptions made in order to adapt the model to the plasma glucose and insulin concentration dynamics associated with the IVTT thus appear to hold, and support is given for the generality of the minimal model in conditions when dynamic changes in plasma glucose concentrations result from an increase in insulin secretion.

The subjects of Study A are probably the largest group of healthy, normal individuals yet assessed by minimal modelling techniques. The mean values of S_I thus obtained compare well with those reported for similar individuals by Chen and co-workers [34], although the mean S_I for the small group they describe [$n=10$, $S_I=0.65 \text{ min}^{-1}\text{U}^{-1}$] is lower than we found, perhaps due to the predominance of subjects with body weights greater than 100% of ideal. Preliminary studies we have undertaken indicate that S_I is highly sensitive to the degree of obesity, and this may account for the narrow range of values of S_I we find in our group of carefully selected normal subjects.

The euglycaemic clamp remains the reference method for measurement of insulin sensitivity, and it is necessary to consider the validity of minimal model-based estimates of insulin sensitivity in relation to comparable clamp-based estimates. This has been examined in detail by Beard and co-workers [35], who compared clamp-derived estimates of S_I with estimates derived from standard IVGTTs and a modified IVGTT protocol in which a bolus injection of tolbutamide was given 20 min after the injection of glucose. This modification was introduced to maximise the insulin concentrations associated with the declining plasma glucose concentration during the IVGTT, and thus minimise the FSD of the estimates of S_I thus obtained. It also separated the phase of the IVGTT during which redistribution of the glucose load throughout the body is occurring from the phase in which plasma glucose concentration falls, and insulin-dependent glucose decay occurs. No correlation was found between the clamp- and standard IVGTT-derived estimates of S_I , whereas a strong correlation was obtained between the clamp- and modified IVGTT-derived estimates. This appeared to be due to the considerably lower FSDs of the estimates of S_I for the modified test compared with the standard test [means: 3.0% vs 17.4% respectively]. In the present study the IVGTTs performed did not include the tolbutamide modification. However, the dose of glucose we used was 0.5 g per kg body weight compared with the 0.3 g per kg dose used by Beard et al. [35] in their standard IVGTT. Also, we have zero-weighted results during the first 3 min of the test. Our protocol thus results in an augmented insulin response compared with the standard IVGTT of Beard et al., [35] and also minimises the effect of redistribution of

the glucose dose. The mean FSD of the estimates of S_I we obtain for both the IVGTT and IVTT in the present study is 4.4%, which compares well with that reported for the tolbutamide-modified test. Our results may therefore be expected to correlate well with those obtained with the modified IVGTT and, by implication, with the euglycaemic clamp.

Support for the validity of the estimates of S_I reported here is provided by the discrimination that is apparent between the estimates of S_I for the healthy normal subjects in Study A, and the estimates in patients in whom diminished insulin sensitivity has previously been demonstrated or would be expected. With regard to Study B, previous studies of the influence of methandienone on carbohydrate metabolism showed that the insulin response to oral or intravenous glucose was dramatically increased to an extent greater than that of any other steroid we have studied [8, 9], an observation consistent with reduced sensitivity to insulin. In Study C, subjects with acromegaly and Cushing's syndrome and patients undergoing other steroid treatments were studied. These conditions and treatments are each known to be associated with insulin resistance [29, 36, 37] and estimates of S_I were consistent with this in all cases.

Minimal modelling of both the IVGTT and IVTT would appear to give a valid measure of insulin sensitivity. The extent to which these methods are used will depend on future experience gained both in research and clinical practice. The value of measuring S_I has not yet been established, primarily because of the lack of a suitable method with which to investigate insulin sensitivity in significant numbers of cases. In its present form, the minimal model methodology requires that the subject under investigation has a sufficient pancreatic insulin response and this would appear to be a limitation where overt diabetes is under investigation. However, it may be that in those cases in which insulin resistance is associated with an augmented insulin response and hyperinsulinaemia, that measurement of S_I will prove to be of greatest value. The long-term consequences of elevated plasma insulin concentrations are only now becoming apparent in relation to coronary heart disease risk [38, 39]. Whether diminished insulin sensitivity predisposes to risk of future morbidity will only be determined if straightforward, effective methods for its measurement are established.

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Dr. N.M. Shennan
The Cavendish Clinic
21 Wellington Road
St. John's Wood
London NW8 9SQ
UK