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Using Glucose Tolerance Tests to Model Insulin Secretion and Clearance

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***Abstract**: The purpose of the studies described in this paper is to develop theoretically and to validate experimentally mathematical compartment models which can be used to predict plasma insulin levels in patients with diabetes mellitus (DM). In the case of Type 2Diabetes Mellitus (T2DM), the C-peptide levels in the plasma were measured as part of routine glucose tolerance tests in order to estimate the prehepatic insulin secretion rates. In the case of Type 1 Diabetes Mellitus (T1DM), a radioactive labelled insulin was used to measure the absorption rate of insulin after a subcutaneous injection of insulin. Both models gave close fits between theoretical estimates and experimental data, and, unlike other models, it is not necessary to seed these models with initial estimates.*

***Keywords**: Compartment modelling, Differential equations, Technetium, Diabetes mellitus, Glucose tolerance tests, Gamma variate.*

Introduction

The two key chemicals in the constant endeavour of the body to produce energy are glucose and insulin. The hormone insulin facilitates the entry of glucose into cells for conversion into energy. Diabetes can be a result of the impairment of the ability of the body to obtain the energy it needs to function properly.

Type 1 Diabetes Mellitus (T1DM) is the name given to that form of the disease where the endogenous production of the insulin in the pancreas is eliminated. Insulin from an external source needs to be provided, usually by subcutaneous injection [1].

In the case of Type 2 Diabetes Mellitus (T2DM) it is usually the quantity or efficacy of insulin that is affected, but there is still secretion of insulin from the pancreas. This form of the disease is usually treated with a combination of diet, exercise and oral agents, though sometimes insulin treatment is also required (in which case both Fig. 1 and Fig. 2 below are related to such cases).

Essentially, the two forms of diabetes are different diseases with similar symptoms. In both cases, diabetes mellitus is a chronic state of excessive concentration of glucose in the blood. The major regulator of glucose concentration in the blood is insulin, a hormone synthesized and secreted by the beta cells of the islets of Langerhans in the pancreas. High blood sugar levels may be due to a lack of insulin and/or to excess of factors that oppose its action and cause insulin resistance.

This imbalance can lead to abnormalities of carbohydrate, protein and lipid metabolism. The major complications of diabetes include characteristic symptoms, the progressive

development of disease of the capillaries of the kidney and retina, damage to the peripheral nerves, and accelerated arteriosclerosis [30]. Owens [12] presents a historical summary of the disease, and Bliss [2] relates the human drama and scientific enterprise behind the discovery of insulin.

The mathematical modelling of subcutaneous insulin clearance and prehepatic insulin secretion is clinically very useful for several reasons. The process itself is quite complicated and the modeling permits us to focus on the salient features. The process is affected by such factors as insulin concentration, the half-life of the insulin, the site, method and type of injection [7]. Knowledge of insulin kinetics also has uses in the study of pre-diabetes [27] and diabetic complications [13, 14], as well as in such therapeutic innovations as pumps [15], jet injectors [26] and bio-synthetic human insulins [12].

This paper develops theoretical models for the secretion of insulin from the pancreas in T2DMs and the clearance of insulin from the injection site in T2DMs. Both models are validated with experimental data, and the theoretical and experimental values are well matched. Both models incorporate the gamma function which has the advantage that there is no need for seeding the computational analysis with initial values, nor is there any sensitivity to the location of sampled points so that the secretion or clearance rates can be estimated at any instant. Furthermore, the parameters of the models have theoretical foundations, unlike the black-box modelling in the more common spline fitting. Although parts of this paper have previously been published this is the first attempt to integrate the whole story.

Subcutaneous Insulin Absorption Rates in T1DM

Insulin absorption rates are altered in the diabetic state [6] and this affects the relation between insulin absorption and the resulting plasma insulin concentration [24]. Insulin absorption can be determined experimentally by labeling the insulin with a radioactive tracer such as I^{125}, and then injecting the labeled preparation subcutaneously; the disappearance of radioactivity from the injection site can then be measured.

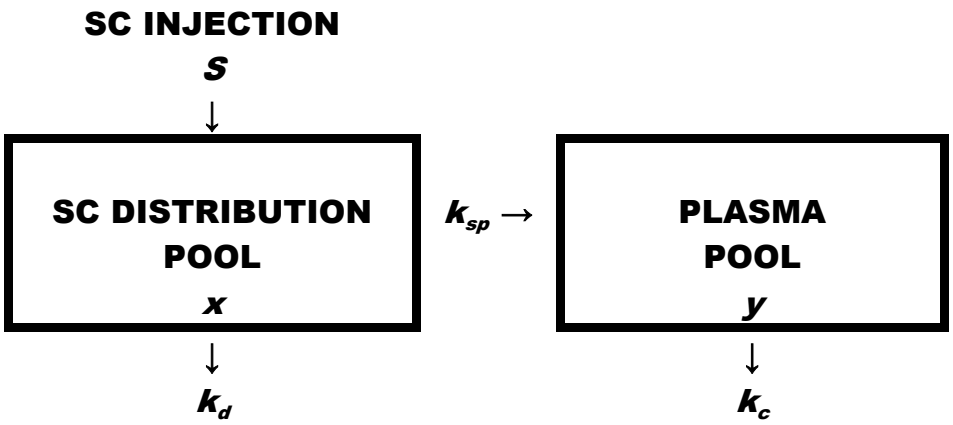

Fig. 1 Compartment model for insulin absorption after a subcutaneous (SC) injection for patients with T2DM

The amount of radioactivity remaining at the injection site can then be plotted against time to obtain a characteristic curve for each type of insulin which is useful for the physician in planning a regimen of insulin doses and types for individual patients.

Studies have shown that I^{125} insulin injected subcutaneously is not normally degraded at the insulin site. It can therefore be assumed that the disappearance of radioactive labeled insulin from the injection site parallels the absorption of the insulin [1]. An absorption rate profile can then be calculated.

Controversy surrounds the residual depot activity: some claim high subcutaneous degradation of insulin [23], while others have shown that the effects of this process are small in normal and most DM subjects [4]. The study of the process can be complicated by the presence of antibodies in DM subjects since this affects both plasma insulin dynamics and the assay procedure. To simplify the study of the absorption process normal subjects are sometimes used with their endogenous insulin secretion suppressed by an infusion of somatostatin [10].

A theoretical model was postulated with a two-compartment (pool) [9] as schematically represented in Fig. 1. A single pool was envisaged for the subcutaneous (SC) distribution of insulin following an injection (bolus) of S international units. It is acknowledged that this is an oversimplification of the biological process since it assumes that all SC insulin is immediately available for transcapillary absorption. The model than assumes a fractional rate of systemic delivery k_{sp} and a degradation rate constant k_d from the SC pool. The plasma pool represents the plasma distribution volume with k_c as the metabolic clearance rate.

The mathematical equations which represent the theoretical model are then

$$\frac{dx}{dt} = S\delta(t) - k_d x - k_{sp} x \tag{1}$$

and

$$\frac{dy}{dt} = k_{sp} x - k_c y \tag{2}$$

where x and y are the amounts of SC insulin in pools 1 and 2 respectively, and $\delta(t)$ is defined by

$$\delta(t) = \begin{cases} 1 & if\ t = 0, \\ 0 & if\ t > 0. \end{cases}$$

The next step is to do something about the parameters. Observation of the appearance of insulin in the plasma shows a rising curve initially [20]. This suggests that insulin is delivered to the plasma pool as

$$y \propto t^a \ (a > 0)$$

or

$$\frac{dy}{dt} = \frac{ay}{t} \ (t \neq 0)$$
$$= k_{sp} x.$$

Equation (2) then becomes

$$\frac{dy}{dt} = \frac{ay}{t} - k_c y. \tag{3}$$

While aware of the danger of becoming slaves to a model, there subsequently seemed to be an experimental justification for these assumptions. Disappearance from the SC site is found from

$$\frac{dx}{dt} = -\left(k_d + k_{sp}\right)x$$
$$= -kx$$

or

$$x = x_0 e^{-kt}. \tag{4}$$

which can be readily linearised. Appearance in the plasma (or clearance from the injection site) is found from equation (3), which can be rewritten as

$$\frac{1}{y}\frac{dy}{dt} - \frac{a}{t} = -k_c$$
$$\frac{d}{dt}\ln y - a\frac{d}{dt}\ln t = -k_c$$
$$\frac{d}{dt}\ln\left(\frac{y}{t^a}\right) = -k_c$$
$$y = y_0 t^a e^{-k_c t}. \tag{5}$$

From equation (5) we have that

$$\frac{dy}{dt} = ay_0 t^{a-1} e^{-k_c t} - k_c y_0 t^a e^{-k_c t} \tag{6}$$

the former term on the right hand side of equation (6) being the non-degraded clearance rate from the SC site and the latter term being the clearance rate from the plasma pool.

External disappearance of I^{125} labelled human soluble insulin (U100) with simultaneous measurement plasma immunoreactive insulin, C-peptide and glucose was used to study this insulin absorption. To assess the relationship between insulin absorption and subcutaneous blood flow the latter was measured by the disappearance of 99M technetium.

Initial studies consisted of five normal subjects studied on four occasions. On the first three study days insulin absorption was measured from the anterior abdominal wall with simultaneous measurement of subcutaneous blood flow from an injection site adjacent to the insulin injection site. The measurement of SC blood flow from this latter site was compared to a simultaneous injection of technetium on the opposite side of the abdominal wall. On the fourth study day subjects received only insulin. Each study day commenced with three basal blood samples at -60, -30 and 0 minutes. The six international units of labeled insulin were injected at time 0 minutes, and thereafter blood samples were obtained at 10 minute intervals for the first hour, every 15 minutes for the second hour, and subsequently every half hour until 6 hours after the injection. External disappearance of the insulin and technetium was measured continuously for the first 2 hours and thereafter for 5 minutes at the time of blood sampling.

Six DM subjects were then subjected to the same regime. Their disappearance results are displayed in Table 1, which tabulates the percentage residual activity of the technetium injected adjacent to the insulin injection site over the first 8 minutes.

Table 1. Measured percentage residual radio activity for 6 subjects during first 8 minutes

Time	Subjects					
(min)	1	2	3	4	5	6
0	100.000	100.000	100.000	100.000	100.000	100.000
1	88.761	89.885	94.262	87.759	92.457	88.265
2	81.613	83.968	85.551	79.320	87.215	78.606
3	72.272	79.510	77.392	71.445	82.852	71.069
4	66.100	72.955	69.424	64.936	78.705	65.968
5	60.142	66.077	64.273	58.378	74.840	60.170
6	53.720	61.891	58.582	52.023	70.523	54.035
7	50.226	59.103	52.886	48.126	66.183	50.549
8	44.617	54.725	47.480	44.693	64.275	45.605

Table 2 shows the results of fitting the data for the same subjects to equation (4) above.

Table 2. Calculated percentage residual radioactivity for 6 subjects during first 8 minutes and the resulting parameter values

Time	Subjects					
(min)	1	2	3	4	5	6
0	98.755	98.125	102.233	97.617	98.259	96.731
1	89.422	91.089	93.036	88.226	93.004	87.992
2	80.971	84.558	84.666	79.738	88.031	79.916
3	73.319	78.494	77.049	72.066	83.323	72.638
4	66.389	72.866	70.117	65.133	78.868	66.023
5	60.115	67.641	63.809	58.867	74.650	60.011
6	54.434	62.791	58.068	53.203	70.658	54.546
7	49.289	58.289	52.844	48.085	66.880	49.579
8	44.631	54.109	48.090	43.459	63.303	45.064
x_0	0.988	0.981	1.022	0.976	0.983	0.970
k	0.099	0.074	0.094	0.101	0.055	0.096
r^2	0.998	0.995	0.998	0.997	0.966	0.966

Some ‘appearance’ results are set out in Table 3. They show the six subjects’ plasma insulin concentration (nmol/l) corresponding to the time vector (min) in the left-most column. Thus the ratio of appearance (A) to disappearance (D) has the form

$$A/D = kt^a e^{-bt} \tag{6}$$

which can also be linearised so that multiple linear regression analysis can be used to fit the data.

Table 3. Measured plasma insulin concentration (nmol/l)

Time (min)	Subjects					
	1	2	3	4	5	6
-60	0.042	0.072	0.030	0.036	0.024	0.024
-30	0.030	0.084	0.030	0.024	0.024	0.024
0	0.030	0.060	0.030	0.018	0.030	0.024
10	0.066	0.054	0.048	0.030	0.030	0.030
20	0.120	0.072	0.078	0.042	0.036	0.072
30	0.144	0.108	0.090	0.072	0.048	0.060
40	0.126	0.114	0.090	0.090	0.042	0.090
50	0.150	0.150	0.102	0.090	0.054	0.108
60	0.150	0.120	0.096	0.072	0.054	0.114
75	0.150	0.108	0.072	0.102	0.060	0.102
90	0.132	0.102	0.090	0.102	0.042	0.084
105	0.138	0.096	0.090	0.096	0.054	0.078
120	0.132	0.078	0.090	0.108	0.048	0.078
150	0.114	0.096	0.084	0.090	0.054	0.096
180	0.108	0.102	0.078	0.066	0.036	0.066
210	0.072	0.090	0.054	0.090	0.030	0.054
240	0.054	0.060	0.066	0.054	0.024	0.048
270	0.048	0.054	0.072	0.054	0.030	0.060
300	0.036	0.048	0.048	0.042	0.024	0.036
330	0.024	0.042	0.030	0.042	0.018	0.024
360	0.030	0.030	0.024	0.042	0.030	0.024

Table 4. Calculated plasma insulin concentration (nmol/l) and appearance/disappearance parameters

Time (min)	Subjects					
	1	2	3	4	5	6
0	2.104	2.118	2.521	2.561	2.820	2.453
10	0.406	0.313	0.299	0.269	0.196	0.291
20	0.274	0.203	0.186	0.164	0.109	0.180
30	0.217	0.159	0.142	0.125	0.079	0.137
40	0.183	0.134	0.118	0.104	0.063	0.113
50	0.160	0.118	0.102	0.091	0.054	0.098
60	0.143	0.106	0.091	0.082	0.047	0.087
75	0.124	0.094	0.080	0.073	0.041	0.076
90	0.110	0.085	0.073	0.067	0.037	0.068
105	0.099	0.079	0.067	0.063	0.034	0.063
120	0.090	0.074	0.063	0.060	0.032	0.058
150	0.076	0.066	0.057	0.056	0.029	0.052
180	0.066	0.062	0.053	0.054	0.028	0.048
210	0.058	0.058	0.050	0.053	0.027	0.045
240	0.052	0.055	0.048	0.053	0.027	0.043
270	0.047	0.054	0.047	0.054	0.028	0.041
300	0.042	0.052	0.046	0.056	0.028	0.040
330	0.038	0.051	0.046	0.058	0.029	0.039
360	0.035	0.050	0.046	0.060	0.030	0.038
k	0.380	0.324	0.404	0.434	0.427	0.433
a	-0.545	-0.646	-0.718	-0.763	-0.903	-0.716
b	-0.098	-0.076	-0.096	-0.105	-0.059	-0.097
r^2	0.989	0.986	0.987	0.987	0.958	0.985

The curvilinear relationship (6) was then fitted and the resulting parameters are listed in Table 4.

Prehepatic Insulin Secretion Rates in T2DM

We base the following model on evidence that insulin and C-peptide are co-secreted in equimolar quantities [19] and that C-peptide is not taken up by the liver [16, 17]. We can then use C-peptide levels to estimate insulin secretion as schematically represented in Fig. 2. This approach was adopted in [5, 28, 29].

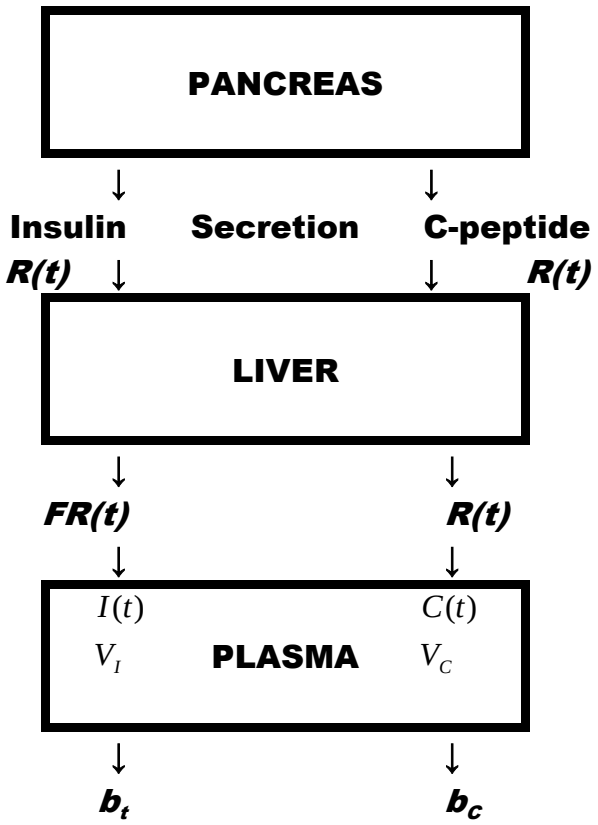

Fig. 2 Compartment model of peptide secretion for patients with non-insulin dependent diabetes mellitus

The main purpose of this part of the study was to determine whether as fasting plasma glucose levels increased the insulin secretion rate decreased in response to a carbohydrate challenge and also whether obese subjects had a lower insulin secretion rate than non-obese subjects.

Suppose *R(t)* is the rate of secretion from the pancreas into the portal vein of both the insulin and the C-peptide in Fig. 2. (1-*F*) is the unknown fractional uptake of insulin by the liver.

To accommodate the initial response by the pancreas to a glucose load, we assumed the rate of secretion of each peptide is directly proportional to the concentration in the plasma and inversely proportional to time with respective coefficients of proportionality a_I and a_C. This is a pragmatic assumption based on experimental observations of results where the glucose challenge is derived from oral glucose tolerance tests (OGTT) and meal tolerance tests (MTT).

$$\frac{dC}{dt} \propto \frac{C}{t}.$$

This assumption was originally prompted by work on the two-pool model for insulin secretion. In this, one pool is conceived as a small compartment available for rapid insulin release, and the other for sustained insulin release tightly coupled to synthesis [18]. Beyond that the use of the assumption leads to consistent results as we shall see. In any case, the hypothesized multiphasic close temporal associations between pulses of insulin secretion and blood glucose levels are the object of considerable debate [10, 16].
We seek $R(t) = dC / dt$. The C-peptide kinetics can then be described by the first order differential equation

$$\frac{dC}{dt} = a_C \frac{C}{t} - b_C C, \tag{7}$$

a solution of which is

$$C = C_0 + At^{a_C} e^{-b_C t}, \tag{8}$$

in which A is a scaling factor.

As we are primarily concerned with the index of secretion a_C, we considered the sensitivity of the model to errors in the parameter a_C. We used an Eulerian approximation to differentiate the natural logarithm of equation (8) to obtain

$$\frac{\Delta a_C}{a_C} = \frac{\Delta a_c \ln t}{a_C \ln t},$$

so that theoretically the relative error in a_C for any given b_C should be a decreasing proportion of the relative error in C-peptide measurement as t increases, though the values for a_C are unduly sensitive to the choice of time points for the secretion phase because $\ln t < t$ and is small for small t (minutes). This is a mathematical limitation of the model and means in practice that agreed times for sampling are necessary for reproducibility and comparisons.

If we differentiate equation (8) we obtain

$$\frac{dC}{dt} = Aa_C t^{a_C - 1} e^{-b_C t} - Ab_C t^{a_C} e^{-b_C t} \tag{9}$$

which is consistent with equation (6) as we would expect. The first term on the right hand side of equation (9) is the secretion term and the second term is the clearance term.

In the following experimental studies all subjects were given an MTT after a 10 hour overnight fast. The meal used is summarized in Table 5. The subjects were allowed 10 minutes to consume the meal.

Table 5. Composition of MTT [8]: A. Made up to 200ml volume with water

Food	Amt (g)	Energy (kcal)	Protein (g)	Fat (g)	Total CHO (g)	Sugars (g)	Starch+ dextrins (g)	Diet fibre
Weetbix	15	51.0	1.71	0.51	10.55	0.92	9.98	1.90
Skimmed milk powder[a]	10	35.5	3.64	0.13	5.28	5.28	0	0
Pineapple juice	250	132.5	1.00	0.25	33.50	33.50	Trace	0
White meat chicken	50	71.0	13.25	2.00	0	0	0	0
Hovis bread	60	136.8	5.82	1.32	27.06	1.44	25.62	2.70
Butter	9	66.6	0.04	7.38	Trace	Trace	0	0
Totals		493.4	25.46	11.59	76.39	41.14	35.60	4.60
%Calories		100	20	20	60			

Pilot Study

As a pilot study we investigated 11 T2DM subjects and 7 non-diabetic subjects, and found the average results in Table 6 following a MTT. The slope is from y_0 to $y_{\max}$ when the clearance action is first perceived.

Table 6. Pilot study parameters for equation (7)

Subjects	N	a_C	b_C	r^2	slope
T2DM	11	4.815	0.052	0.87	0.005
Non-DM	7	4.058	0.063	0.92	0.027

After the overnight fast, the subjects were admitted to a metabolic unit, where they remained on bed rest throughout the study; smoking was not permitted. Mixed venous blood samples were taken from a forearm vein at 08.30 h and immediately prior to the administration of the MTT at 09.00 h, and then at 30 minute intervals for 4 hours.

The C-peptide data were then fitted using multiple linear regression of Y on x and t. Table 7 shows the values of the parameters for the 11 diabetic and 7 non-diabetic subjects in the pilot study. The most obvious difference between the two groups is, not surprisingly, in the slope, though this is really a side-issue in the present study, particularly in the pilot study. However, it does illustrate that the model picks up the sharper response of the non-diabetic subjects to the glucose load.

Table 7. Individual subjects in pilot study

T2DMs	a	b	r^2	slope	Non-DMs	a	b	r^2	slope
1	1.412	0.008	0.94	0.001	1	1.590	0.024	0.78	0.014
2	8.027	0.075	0.92	0.001	2	3.210	0.058	0.98	0.026
3	2.523	0.021	0.93	0.001	3	3.791	0.047	0.94	0.019
4	2.080	0.026	0.93	0.001	4	2.561	0.032	0.90	0.020
5	1.789	0.024	0.73	0.009	5	11.533	0.120	0.91	0.061
6	4.711	0.105	0.60	0.004	6	1.938	0.118	0.95	0.024
7	8.770	0.067	0.82	0.014	7	3.780	0.045	0.96	0.022
8	8.630	0.086	0.87	0.002					
9	9.104	0.089	0.89	0.004					
10	3.519	0.036	0.94	0.007					
11	2.395	0.026	0.95	0.006					

As an example of the goodness-of-fit, the measured (m) and the calculated (c) C-peptide levels for the T2DM subjects 1 and 6 are shown in Table 8. A more sophisticated analysis of the issues in goodness-of-fit is discussed in some detail in [22].

Table 8. Comparisons at specific sampling times for T2DMs 1 and 6

	time (min)	0	15	30	45	60	75	90	120	150	180
#1	m	.11	.14	.18	.19	.24	.26	.37	.43	.39	.34
	c	.11	.14	.17	.21	.25	.27	.30	.33	.35	.36
#6	m	.37	.45	.48	.52	.42	.42	.41	.39	.41	.24
	c	.37	.39	.39	.38	.37	.37	.37	.37	.37	.37

Main study

The pilot study was carried out at the Prince of Wales Hospital in Sydney, Australia. The main study, which follows, was performed at the University Hospital in Cardiff, Wales, after the model was discussed with researchers from the Radcliffe Infirmary in Oxford, England, where my wife who has T1DM was a patient.

In this study there were 235 T2DM patients. All patients had normal kidney and liver function tests, though as an indication of glycaemic control the glycosylated haemoglobin (HbA1) concentration of the patients varied from 6.7 to 19.3% (mean 11.6, SD 2.5%). (For comparison, a normal range is 5.5-7.8%). The DM subjects were sub-divided into three subgroups labeled, ‘mild’, ‘moderate’ and ‘severe’, as defined in Table 9.

For later clinical work, as well as to test the model, the patients were also divided into obese and non-obese subgroups according to body mass index (BMI=body mass/height^2). It was used because it partly accounts for the distribution of the body mass. A BMI<26.5 kg/m^2 is commonly taken as non-obese [13]. In addition, 56 normal subjects of similar age range and no family history of DM were studied.

Table 9. Classification of subjects into MTT and BMI subgroups

	MTT				
BMI	Normal	Mild	Moderate	Severe	Total
Non-obese	32	19	25	12	88
Obese	5	39	40	23	107
Total	37	58	65	35	195
BSL (mmol/l)	4-7	7-10	10-13	>13	

Table 10 lists a comparison of the four sub-groups in terms of

- secretion indices (a_C),
- clearance indices (b_C),
- the parameter $\beta(=-\ln A)$,
- peak concentration (C_m'),
- time for peak concentration (t_m),
- slope of the peak concentration versus time for peak concentration (λ),
- peak secretion rate (r_p),
- time for peak secretion rate (t_p), and
- the total amount secreted (S).

Table 11 sets out similar comparisons between the obese and the non-obese groups.

Table 10. Comparison of normal (A), mild (B), moderate (C), severe (D)

Variable	Sub-group	Means	Significant differences between the variances	Significant differences between the means	Significant pair wise differences
a_C	A	1.84	No	Yes	A-B
	B	2.52			A-C
	C	2.64			
	D	2.57			
b_C	A	0.0274	No	Yes	A-D
	B	0.0224			
	C	0.0218			
	D	0.0189			
β	A	5.60	No	Yes	A-B
	B	9.40			A-C
	C	10.26			A-D
	D	10.50			
$C_m^{/}$	A	1.67	Yes	Significant	
	B	1.34		difference	
	C	0.99		between the	
	D	0.70		medians	
t_m (min)	A	67.9	No	Yes	A-B
	B	123.9			A-C
	C	129.5			A-D
	D	140.9			B-D
λ	A	0.0286	Yes	Significant	n/a
	B	0.0112		difference	
	C	0.0081		between the	
	D	0.0061		medians	
r_p	A	0.0719	Yes	Significant	n/a
	B	0.0365		difference	
	C	0.0266		between the	
	D	0.0194		medians	
t_p (min)	A	35.96	Yes	Significant	n/a
	B	65.85		difference	
	C	71.91		between the	
	D	75.07		medians	
S	A	5.68	Yes	Significant	n/a
	B	5.44		difference	
	C	3.94		between the	
	D	3.07		medians	

Table 11. Comparison of obese (A) versus non-obese (B)

Variable	Sub-group	Means	Ratio of variances=1	Difference between the means=0	Difference between the medians=0
a_C	A	2.406	Yes	Yes	Yes
	B	2.481			
b_C	A	0.0200	No	No	No
	B	0.0256			
β	A	9.185	No	Yes	Yes
	B	9.131			

$C_m^{/}$	A	1.191	Yes	Yes	Yes
	B	1.176			
t_m	A	128.45	No	No	No
	B	105.47			
λ	A	0.0104	No	No	Yes
	B	0.0152			
r_p	A	0.0321	No	Yes	Yes
	B	0.0399			
t_p	A	65.12	Yes	Yes	Yes
	B	64.90			
S	A	4.676	Yes	Yes	Yes
	B	4.413			

Conclusion

For reasons of brevity and because the main goal was to establish the plausibility of the modelling process, the clinical implications for interpretation of the GTT or in infusion of insulin [31] are not explored here.

One final point about the 'gamma variate' model presented here relates to the 'area under the curve' (AUC). While not denigrating the Tai model for the total area under metabolic curves [25], nor denying its validity, the gamma variate model can also be used to approximate the AUC, as well as describe these curves functionally with estimates of their characteristic parameters and to separate their secretion and clearance phases as we have shown.

If we integrate the equation for *S*, and if a long interval is considered, then the total AUC is given by

$$S = Aa_C \int_0^\infty t^{a_C - 1} e^{-b_C t} dt$$
$$= a_C A \Gamma(a_C) / b_C^{a_C}$$

where Γ represents the gamma function. For the same long time interval, this may be shown to equal the total amount cleared. (For short time intervals, the total amount cleared may be considerably less than the total amount secreted.)

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