Gamma Variate Analysis of Insulin Kinetics in Type 2 Diabetes

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Abstract: *The purpose of this paper is to offer a mathematically simple and theoretical based alternative model to existing techniques for the estimation of beta cell secretion of peptides. This is achieved by applying gamma variate analysis rather than cubic spline fitting techniques to measured insulin and glucose levels. The resulting fits between experimental and theoretical data are excellent. Cubic spline fitting methods can also give excellent results, though to some extent this is an artefact of these methods, which by their very nature give smooth fitting to experimental or plotted points. Unfortunately, spline fitting is very sensitive to the choice of knot points, the interval of measurement, and the accuracy of initial estimates of the parameters.*

Keywords: *Type 2 diabetes mellitus, Glucose clamps, C-peptide, Insulin, Gamma-variate, Coefficient of determination.*

Introduction

Methods for the quantification of beta-cell sensitivity to glucose (hyperglycemic clamp technique) and of tissue sensitivity to insulin (euglycemic insulin clamp technique) are described by DeFronzo et al. [6]. In these, the plasma glucose concentration is acutely raised above basal levels by a priming infusion of glucose. The desired hyperglycemic plateau is subsequently maintained by adjustment of a variable glucose infusion, based on the negative feedback principle. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of glucose metabolism. Under these conditions of constant hyperglycemia, the plasma insulin response is biphasic with an early burst of insulin release during the first six minutes followed by a gradually progressive increase in plasma insulin concentration.

In the euglycemic insulin clamp technique, the plasma insulin concentration is acutely raised and maintained by a prime-continuous infusion of insulin. The plasma glucose concentration is held constant at basal levels by a variable glucose infusion using the negative feedback principle. Under these steady-state conditions of euglycemia, the glucose infusion rate equals glucose uptake by all the tissues in the body and is therefore a measure of tissue sensitivity to exogenous insulin.

The purpose of this study is to consider a mathematical model of peptide kinetics. The technique of Vølund et al. [11] offers a relatively simple approach to the estimation of beta-cell secretion of peptides by applying cubic spline fitting techniques to measured peptide levels. The resulting fits between experimental and theoretical data are excellent, but the method is very sensitive to various “shrewd guesses”, especially the relative accuracy of the

initial measurements of the parameter which can be subject to computational ill-conditioning problems [3].

Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (DM2) is also known as Non Insulin-Dependent Diabetes Mellitus (NIDDM) and adult-onset diabetes. However, this type can eventually lead to insulin treatment and also is now appearing in many children so those terms are no longer appropriate. Many people develop Type 2 diabetes mellitus without an accompanying drop in insulin levels (at least at first) [8]. In many cases, the problem appears to be a failure to express a sufficient number of glucose transporters in the plasma membrane (and T-system) of their skeletal muscles [1].

Insulin is a small protein consisting of an alpha chain of 21 amino acids linked by two disulfide (S-S) bridges to a beta chain of 30 amino acids.

Beta cells have channels in their plasma membrane that serve as glucose detectors. Beta cells secrete insulin in response to a rising level in circulating glucose ("blood sugar"). Insulin affects many organs. It stimulates skeletal muscle fibres, acts on liver cells; it acts on fat (adipose) cells to simulate the uptake of glucose and the synthesis of fat; it acts on cells in the hypothalamus to reduce appetite. In each case, insulin triggers these effects by binding to the insulin receptor – a trans-membrane protein embedded in the plasma membrane of the responding cells.

The alpha cells of the islets, on the other hand, secrete glucagon, a polypeptide of 29 amino acids. Glucagon acts principally on the liver. The physiological significance is that glucagon functions to maintain a steady level of blood sugar level between meals. Many patients with DM carry glucagon tablets as a precaution against hypo-glycaemic reactions ("hypo's").

The delta cells of the islets secrete somatostatin which has a variety of functions. Taken together, they work to reduce the rate at which food is absorbed from the contents of the intestine. Somatostatin is also secreted be the hypothalamus and by the intestine. It is one of the drugs which may be used as part of the treatment of DM2.

The gamma cells of the islets secrete pancreatic polypeptide, which reduces appetite. Failure to achieve glycaemic stability can result in a variety of side effects in patients with diabetes [10], particularly retinopathy, as diabetes is the major cause of adult blindness in many countries [7].

Insulin kinetics

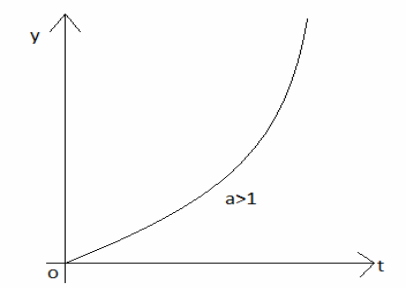
Islet beta-cells express both insulin receptors and insulin-signalling proteins. Recent evidence from rodents *in vivo* and from islets isolated from rodents or humans suggests that the insulin signalling pathway is physiologically important for glucose sensing. Bouche et al. [4] evaluated whether insulin regulates beta-cell function in healthy humans *in vivo*. Their studies demonstrated that insulin potentiates glucose-stimulated insulin secretion *in vivo* in healthy humans. In addition, hyperinsulinemia increases C-peptide clearance, which may lead to modest underestimation of beta-cell secretory response when using these methods during prolonged dynamic testing.

The model

An examination of insulin and C-peptide curves to glucose loads shows that in the initial phase they have an exponential shape, that is,

$$y = At^a \tag{1}$$

for some constant of *A*.



If we assume that this phase is principally due to secretion, then the secretion rate is

$$\frac{dy}{dt} = aAt^{a-1} = \frac{a}{t}y \tag{2}$$

and we have a secretion coefficient *a/t.*

The experimental curves then go to a relative maximum turning point followed by a period of decay. We postulate a clearance coefficient, *b*, so that the clearance rate is

$$\frac{dy}{dt} = -bt \tag{3}$$

The net result would be that the rate of change of peptide can be represented by

$$\frac{dy}{dt} = \frac{a}{t}y - by \tag{4}$$

This can be compared with the model Vøland et al. [11] for insulin and C-peptide metabolism which is

$$\frac{dy}{dt} = fr(t) - by \tag{5}$$

where *f* is related to the fraction not taken up by the liver (*f* = 1 for C-peptide), *r*(*t*) is the secretion rate and *b* is a first order elimination constant ($b = k_1$ and k_c for insulin and C-peptide respectively).

A solution of (5) is given by

$$y = y_0 + Ct^{a-1}e^{-bt} \tag{6}$$

in which y_0 is a base-level for the peptide in question, and C is a numerical scaling coefficient. That it satisfies the differential equation can be readily verified:

$$\frac{dy}{dt} = aCt^{a-1}e^{-bt} - bCt^a e^{-bt} = \frac{a}{t}Ct^a e^{-bt} - bCt^a e^{-bt} = \frac{a}{t}y - by$$

as required.

Furthermore a most important point in practice is that this solution can be linearized:

$$y - y_0 = Ct^a e^{-bt}$$
$$\ln(y - y_0) = \ln C + a\ln t - bt$$

which has the form

$$Y = c + ax - bt \tag{7}$$

in which $Y = \ln(y - y_0)$, $c = \ln C$, and $x = \ln t$. This linearization is the mathematical reason that this model does not need to be seeded with an initial guess.

Results

A fit can now be carried out using a multiple linear regression of Y on x and t [9]. When we do so we get very good fits in a least-squares sense as measured by r^2, the coefficient of determination. a can be regarded as an index of secretion. The slope from y_0 to $y_{\max}$ or the slope to the first point of inflection (when the clearance action is first perceived) could be used as indices of secretion: it depends how finely tuned such an index should be. The former slope was used in the following results.

For the subjects in the study the following average results have been obtained with the model for the C-peptide levels following a meal test.

Table 1

	N	*a*	*b*	r^2	*slope*
Diabetics	11	4.815	0.052	0.87	0.005
Non-diabetics	4.058	0.063	0.92	0.027	4.058

The advantages of this model are that the parameters have a theoretical foundation (unlike the black-box modelling in spline-fitting), the experimental and theoretical values fit well, there is no need for seeding the computational analysis with initial values, nor is there any sensitivity to the location of sampled points. $(a/t)y$ can be used to measure the secretion rate at any instant. For instance, Tables 2 and 3 show the values for the relevant parameters for 11 diabetic and 7 normal subjects respectively, following a meal tolerance test. This is based on their C-peptide levels. Data originally came from Professor D. R. Owns, *CBE* now of the School of Medicine, the University of Cardiff, Wales, and Professor S. Colagiuri of the Faculty of Medicine, the University of Sydney, Australia.

Table 2

Diabetics	*a*	*b*	r^2	*slope*
1	1.412	0.008	0.94	0.001
2	8.027	0.075	0.92	0.001
3	2.523	0.021	0.93	0.001
4	2.080	0.026	0.93	0.001
5	1.789	0.024	0.73	0.009
6	4.711	0.105	0.60	0.004
7	8.770	0.067	0.82	0.014
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

Table 3

Non-diabetics	*a*	*b*	r^2	*slope*
1	1.590	0.024	0.78	0.014
2	3.210	0.058	0.98	0.026
3	3.791	0.047	0.94	0.019
4	2.561	0.032	0.90	0.020
5	11.533	0.120	0.91	0.061
6	1.938	0.118	0.95	0.024
7	3.780	0.045	0.896	0.022

Sensitivity

As an example for the goodness-of-fit the measured (*m*) and calculated (*c*) C-peptide levels (p mol/ml) for two extreme subjects (1 and 6) are shown in Table 4.

Table 4

Subject 1										
Time (min)	0	15	30	45	60	75	90	120	150	180
m	0.11	0.14	0.18	0.19	0.24	0.26	0.37	0.43	0.39	0.34
c	0.11	0.14	0.17	0.21	0.25	0.27	0.30	0.33	0.35	0.36
Subject 6										
Time (min)	0	15	30	45	60	75	90	120	150	180
m	0.37	0.45	0.48	0.52	0.42	0.42	0.41	0.39	0.41	0.24
c	0.37	0.39	0.39	0.38	0.37	0.37	0.37	0.37	0.37	0.37

Theoretically, the relative error in *a* for any given *b* should decrease as *t* increases:

$$\Delta Y = Y(t^{\Delta a} - 1),\ Y = y - y_0$$

$$(\frac{\Delta Y}{Y})^{1/a} = t^{\frac{ba}{a}}(1-1/t^{\Delta a})^{1/a} \doteq t^{\Delta a/a}$$

$$\frac{\Delta a}{a} = \frac{\ln(\Delta Y / Y)}{a \ln t},\ t \neq 0 \tag{8}$$

In practice, the coefficient *a* remains within about 10% of its original value if the readings at 45 minutes are omitted. More studies need to be done to establish and compare ranges for the parameters within and between subjects, and distinguished by body mass index [2].

Conclusion

The next stage in the development of the model is to incorporate the Vølund insulin/C-peptide relationship to estimate pre-hepatic insulin secretion rates by taking the ratios of the secretion terms in (6) for the two peptides:

$$f = a_I I(t) / a_C C(t) \tag{9}$$

The advantages of this model are that the parameters have a theoretical foundation (unlike black-box modelling [5]), the experimental and theoretical values fit well, there is no need for seeding the computational analysis with initial values, nor is there any sensitivity to the location of sampled points. (*a/t*)*y* can be used to measure the secretion rate at any instant.

Another advantage of this model is that the time to reach maximum *y* can be readily calculated. This may be more useful than determining *y* max from the largest value of *y* corresponding to the selected time points, since *y* max might well occur between these points. Since *dy/dt* is zero for *y* max, this occurs when *t* = *a/b*. If a single index of secretion were needed, the slope from y_0 to *y* max might be considered.

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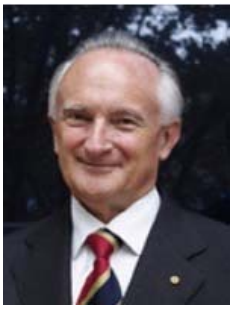

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He enjoys reading, walking, theatre, number theory, and thoroughbred racing.