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Exploring biology through mathematical models: glucose transporter trafficking

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In order to make use of and regulate glucose in the blood stream, the body alters the rate of passage of glucose into and out of cells. For example, in moments of high physical exertion, muscle cells require increased amounts of energy to fuel their increased activities and take more glucose from the bloodstream. Another example is the increased uptake of glucose by fat cells after a meal in an effort to store the transitory increase in available energy. These cells can do this since they have special machines known as glucose transporter proteins on their surfaces, that act as gateways through which glucose can cross the otherwise impermeable layer covering the cell known as the plasma membrane. Cells change the amount of glucose going in or out by regulating the number of such gateways exposed on the surface of the cell. Gateways removed from the surface are no longer exposed to glucose outside the cell and this reduces how much glucose the cell takes in.

The cell manages to do this by pulling parts of the plasma membrane inwards into the cell, forming spherical compartments known as vesicles. The glucose transporters which exist on the membrane are then taken inside the cell along with the vesicles, as seen diagrammatically in Figure 1. The reverse process occurs when the cell requires more glucose: it may either make more transporters (a very slow process) and/or cause pre-existing transporters to move to the surface where they may increase the rate of glucose transportation into the cell. The reader is encouraged to consult an earlier article (A.C.F. Coster, Modelling the movement of vesicles in cells, *Parabola Incorporating Function*, **46**(3), 2010) for further details on vesicle movement. One such gateway is glucose transporter type 4 (GLUT4) which is responsive to chemical signals from the bloodstream, specifically to the hormone insulin.

We are particularly motivated to understand the glucose transporter system because it is involved in a very common disease–diabetes mellitus. In type 2 diabetes, cells no longer respond as they should to insulin signals and so the glucose transportation system fails to respond to the body's requirements. For example, if blood glucose levels rise after a meal but there are too few GLUT4 at the surface of cells to transport the available glucose then this may result in long term elevation of blood glucose, which is symptomatic of diabetes.

In this article, we'll see how simple mathematical modelling of this system may be carried out, evaluated and to some degree refined with more complex models. We

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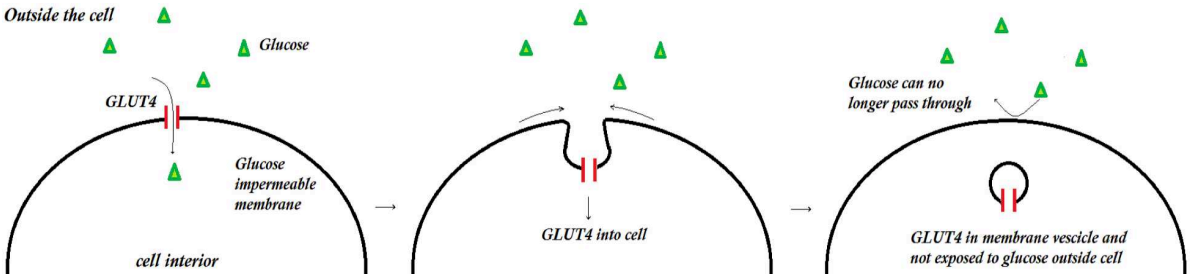

Figure 1: A (very) simplified diagram of a reduction in glucose uptake, achieved by decreasing the number of GLUT4 at the plasma membrane.

will also make adjustments to this model that are informed by biological knowledge of the glucose transporter system. It is hoped that the reader will appreciate a fruitful interplay between maths and biological research, that biology presents interesting phenomena that can be understood from a mathematical viewpoint and how maths may suggest routes for future biological investigation.

The empirical observations: transition and steady state experiments

For the purposes of this study, two experimental procedures were investigated. The first procedure is called the transition experiment and the second procedure is called the steady state experiment. In the transition experiment, the surface concentration of GLUT4 of the cells were measured as a function of time. The cells were first observed in a steady base state, that is, they were observed when no insulin was present. After the base state was measured, the cells were exposed to a predetermined quantity of insulin and the change in surface GLUT4 was recorded as a function of time. Eventually, it was observed that the surface GLUT4 settled into a new steady state which we'll call the insulin stimulated state. Note that these steady states show *large scale* constant levels of surface GLUT4; this does not imply that the movement of GLUT4 from surface to interior stopped but rather that the cell was in *dynamic equilibrium* where the rates of GLUT4 to and from the surface were just balanced to cancel each other out and have a zero net rate of trafficking.

In the steady state experiments, the cells were already in one of the two steady states (base or insulin stimulated) and the surface GLUT4 was labelled with a chemical so that any GLUT4 exposed to the cell surface glowed. As the cell was in a state of dynamic equilibrium, GLUT4 trafficking still occurred and therefore labelled GLUT4 from the surface was moved into the cell interior and both labelled and unlabelled GLUT4 made it to the surface. As this process continued, the total concentration of labelled GLUT4 increased until all the GLUT4 participating in the cell's cycling pathway was labelled. In this experiment, the total concentration of labelled GLUT4 with time was recorded.

Throughout, we will illustrate our discussions with comparisons to data taken from

these two modes of experiment conducted on two different types of cells: pre-fat cells and fat cells. Pre-fat cells are a special type of cell in an early stage of development that under appropriate conditions can change [2] into fat cells. Fat cells are cells that primarily function to store energy as fat (more generally, lipids). A proportion of such lipids are made as a consequence of the way glucose is processed in cells.

Differential equations: a common tool for modelling

We now take a little diversion from our discussion of biology to familiarise ourselves with common mathematical tools, techniques and objects of study which we shall make use of in our modelling namely, differential equations (DEs). The reader too, may have already been exposed to this notion during senior high school mathematics: a differential equation is a way to express the change of some quantities with respect to others, according to a given rule describing this change and naturally, they involve the familiar notion of the derivative. The differential equation is often the tool of choice employed in modelling the natural world because such problems often involve relationships between various changing quantities. Intuitive examples include quantities that change with time: distance, population growth, cost, consumption of resources and so on. A goal that we often aim towards when dealing with differential equations is the obtaining of a *solution* to the equation. This solution is not a set of values as in the case of elementary algebraic equations with which we are all familiar (e.g. $x^2 = 1 \implies x = \pm 1$) but yet another equation–this time without referring to derivatives, that explicitly describe the relationship between two (or more) variables.

Regardless of the physical nature of the variables, there may be some unifying *structure* that underlies their dynamics. That is, they may change according to very similar rules. If we are able to mathematically respond to and understand these general rules we may be able to obtain solutions to an incredibly diverse range of specific problems that arise in many and varied fields. This 'line of thinking' so to speak is very common and powerful in maths and is known as a process of *abstraction*. The reader is encouraged to frequently reflect on its application as (s)he will no doubt meet with it repeatedly throughout future studies.

Compartmental modelling

In order to understand the trafficking of GLUT4 in the cells, we need to quantitatively describe the ongoing actions and processes in precise mathematical terminology. This is the process of constructing mathematical representations or *models* of the phenomenon. A guiding principle that we will observe throughout this process is commonly known as Ockham's razor: that we should favour the simplest model possible that sufficiently describes the observed behaviour of our system of interest. There

[2] The conventionally accepted biological term for such a process is 'differentiation' but is avoided to prevent confusion in a mathematical context.

is good reason to adopt this principle–as by making fewer assumptions we are able to avoid postulating unnecessary complexity and the difficulties that arise in testing or confirming them. Further, simpler models are more mathematically tractable than complex ones; a model may still have utility even though it does not entirely reflect our phenomenon of interest, as long as it is an adequate reflection of specific aspects of the phenomenon. This ongoing tension between detail and tractability is present in any modelling task and it is up to the judgment of the individual as to what details are necessary and relevant to include in any mathematical representation.

For the types of situations like those described here, a technique called compartmental modelling is often fruitfully applied. The simplest illustration of compartmental modelling is a single compartment outflow as depicted in Figure 2.

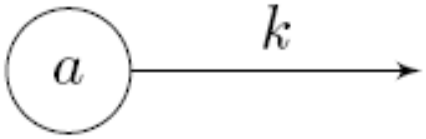

Figure 2: A simple outflow diagram

Mindful that there is no inflow term and a single outflow, the differential equation describing this process is

$$\frac{da}{dt} = -ka. \tag{1}$$

where a is the concentration of the substance, t is time and k a constant. We note that the rate of outflow from each compartment is proportional to the concentration of the substance present in the compartment at that time. This is termed a 'first order' model. Similarly the rate of inflow into a compartment is proportional to the concentration of the substance contained within the individual source compartments from which it is trafficked.

The differential equation (1) should be familiar to the reader, having studied senior mathematics in high school. It is a *separable* differential equation and other than the trivial solution, $a = 0$, can be solved as follows:

$$\int \frac{da}{a} = -k \int dt \implies \ln a = -kt + c.$$

Assuming initial condition $a(0) = a_0 > 0$, we have $c = \ln a_0$ and so $a(t) = a_0 e^{-kt}$.[3] We therefore find that an exponential may arise and indicate processes of outflow (or inflow if the constant k is negative, equivalent to the arrow entering rather than leaving the compartment) and it is reasonable to expect exponentials to arise in more complicated compartmental models. The reader should also note that the parameter k here

[3]Other techniques that can be used to investigate the differential equations in this article are the integrating factor technique and method of undetermined coefficients. The reader is encouraged to refer to a standard text on Differential Equations for details on these methods.

affects rate, with increasing k resulting in faster outflow, in fact see that if $k = 0$ then $a(t) = a_0$ indicating no outflow at all.

Going one step further, we can investigate the case of two compartments as seen in Figure 3.

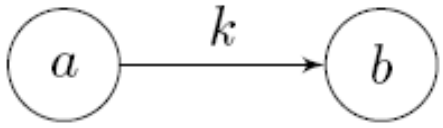

Figure 3: A simple two compartment diagram

For compartment a, we have the same differential equation (1) with exactly the same solution. Compartment b receives the outflow from a and so $\frac{db}{dt} = ka$. Assuming that there is a_0 amount of substance in a and b is empty at $t = 0$, we see that the amount at any given time is

$$b(t) = a_0 - a(t) = a_0 - a_0 e^{-kt} = a_0\left(1 - e^{-kt}\right). \tag{2}$$

We again see that exponentials are involved in describing the behaviour of such systems. For a final toy example, we allow drainage from compartment b, depicted in Figure 4.

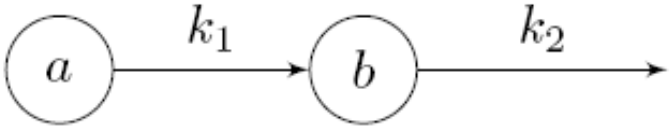

Figure 4: A simple two compartment diagram with drainage

If we assume that initially b is empty, and there is a_0 in a, we arrive at the differential equations

$$\frac{da}{dt} = -k_1 a, \ \frac{db}{dt} = k_1 a - k_2 b, \tag{3}$$

and see then that while the solution for compartment a is unchanged, the solution for b is:

$$b(t) = \frac{k_1 a_0}{k_1 - k_2}\left(1 - \mathrm{e}^{-t(k_1 - k_2)}\right)\mathrm{e}^{-k_2 t}. \tag{4}$$

The reader can verify this by substituting the solutions for a and b back into the differential equations (3). Here, we have assumed implicitly that $k_1 \neq k_2$. If instead however, the rate constants are equal (say $k_1 = k_2 = k$) we see that $\frac{db}{dt} = k(a - b)$ which with the same initial condition $b(0) = 0$, yields the solution: $b(t) = a_0 k e^{-kt} t$.

This demonstrates that in a solution describing the behaviour of flow in a compartment, a single exponential by itself may reflect the combination of a number of effects or multiple processes, some of which may be observationally indistinguishable from a simpler compartment model. This may be because some rate constants have the same value (as above). Alternatively there may be different compartment models (as we shall see, we are not restricted to model just simple sequential flows) with different connections and flows that, depending on the value of rate constants may give rise to seemingly simple solution forms. In such cases a single exponential may be an adequate description of overall behaviour system, albeit not a true reflection of the internal structure of the system. The motivated reader may wish to seek out other examples of this.

This discussion illustrates firstly, the tension between detail and tractability as it is much easier to work with a simple forms than more complex ones and secondly, the notion that it is not possible to confirm with absolute certainty that a mathematical representation is a true description of a physical system. Here, we only deal in possible explanations and not certainties. However, this is not to say, that we are unable to rule out with certainty some models as shall be seen further in this article.

This modelling can be extended to multiple compartments and multiple connections. For each compartment in the model, the total rate of change is equal to the degree of inflow less the outflow per unit time, that is (if the processes are first order and the substance is conserved):

$$\frac{da}{dt} = \underbrace{\sum_{i} (k_i b_i)}_{inflow} - \underbrace{\sum_{j} k_j a}_{outflow} \text{ where } \begin{cases} a & \text{amount of GLUT4 in } A \\ b_i & \text{amount of GLUT4 in source compartments } B_i \\ k & \text{rate constants between compartments.} \end{cases} \tag{5}$$

A model of GLUT4 recycling

For our system we begin with the simple assumption that the total amount of GLUT4 in a cell is constant. We are interested in tracking the changes in the concentration of GLUT4 present at the surface of the cell, in the plasma membrane. It is therefore intuitive to conceptually divide the cell into two 'compartments': the plasma membrane and the cell interior. We can think of the trafficking of GLUT4 as the ongoing flow between these two compartments. We can alter the concentration of GLUT4 at the surface by changing the respective rates of flow from one compartment to the other; as shown in Figure 5. Since we only have two compartments here plasma membrane GLUT4 levels, p, change as a result of the outflow rate, k_{ex}, from the interior of the cell and proportional to the concentration of GLUT4 in the interior, x, and also the inflow rate, k_{en}, (flow from P to X) and proportional to the concentration of GLUT4 at the plasma membrane, p. The rate of change in x follows similarly. We can express this as a pair of differential equations as shown,

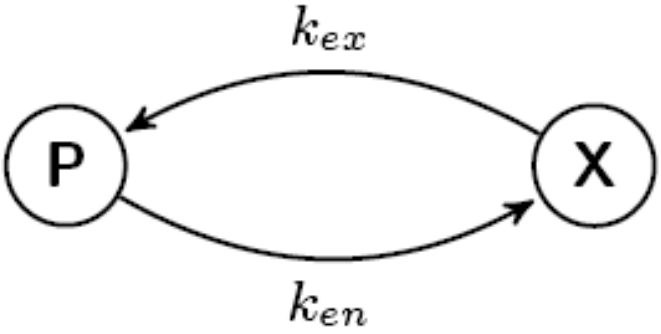

Figure 5: A diagram of the two compartment model. P represents the plasma membrane on the cell surface and X represents the cell interior. k_{ex} is the outflow rate to the plasma membrane and k_{en} is the inflow rate to the interior.

$$\frac{dp}{dt} = k_{ex}x - k_{en}p \text{ and } \frac{dx}{dt} = k_{en}p - k_{ex}x. \tag{6}$$

As the reader will notice, the presence of additional compartments and rates just required additional differential equations of the form (5) to be included in the system. This demonstrates the expandable and modular nature of compartmental modelling and the ease with which models can be further extended. We will now use this model to predict the behaviour of the system in the steady and transition states. We can then evaluate how appropriate the model is by comparing our predictions with the data obtained in the experiments. Empirically, we can estimate the initial level of surface GLUT4 by measuring the initial level of labelled surface transporter instantaneously upon exposing the cells to the glowing chemical tag. That is $p(0) = p_0$, for some estimated p_0. We assume that there are constant levels of GLUT4 in the entire system so this total, $T = p + x$, and hence

$$\frac{dp}{dt} = k_{ex}x - k_{en}p = k_{ex}(T - p) - k_{en}p.$$

This (separable) differential equation, coupled with the initial condition above yields the solution:

$$p(t) = \frac{k_{ex}\,T}{k_{en} + k_{ex}} + \mathrm{e}^{-(k_{en}+k_{ex})t}\left[p_0 - \frac{k_{ex}\,T}{k_{en} + k_{ex}}\right]. \tag{7}$$

The reader is encouraged to verify this by also finding $x(t)$ and substituting these back into the original differential equations (6).

In the steady state, the proportions of GLUT4 do not fluctuate (although the individual GLUT4 are moving) and therefore

$$\frac{dp}{dt} = \frac{dx}{dt} = 0.$$

Hence

$$k_{ex}x - k_{en}p = 0.$$

Since $p = T - x$:

$$x = \left(\frac{k_{en}}{k_{en} + k_{ex}}\right) T \text{ and } p = \left(1 - \frac{k_{en}}{k_{en} + k_{ex}}\right) T = \left(\frac{k_{ex}}{k_{en} + k_{ex}}\right) T.$$

We see therefore, that the steady state concentrations of GLUT4 are completely determined by the value of the rate constants. We denote steady state proportions as follows:

$$S_x = \frac{k_{en}}{k_{en} + k_{ex}} \text{ and } S_p = \frac{k_{ex}}{k_{en} + k_{ex}}. \tag{8}$$

This sheds light on the structure of our solution for $p(t)$, indeed $\lim_{t\to\infty} p(t) = \frac{kex\ T}{ken+kex} = S_p T$.

In the case of the steady state experiment, to track the change in total labelled GLUT4 with time, we need to introduce some extra terms to account for and distinguish between labelled and unlabelled GLUT4. We therefore divide GLUT4 into labelled and unlabelled forms, denoting subscript u for unlabelled and ℓ for labelled so that $x = x_u + x_\ell$ (since at any given time, there may be both labelled and unlabelled GLUT4 within the cell) and $p = p_\ell$ (since all GLUT4 on the membrane is instantaneously labelled when they are exposed to the glowing labelling marker at the surface). Denoting $L = x_\ell + p_\ell$ we note that $T = L + x_u$. Since the GLUT4 cannot become unlabelled and eventually all GLUT4 recycles to the plasma membrane, the amount of total labelled GLUT4 must increase until all GLUT4 are labelled. The only source of unlabelled GLUT4 resides in the interior of the cell and is labelled once it is trafficked from interior to the membrane with rate constant k_{ex}. Therefore,

$$\frac{dL}{dt} = k_{ex}x_u = k_{ex}(T - L) = k_{ex}T - k_{ex}L.$$

We see that now we end up with a first order linear differential equation with constant rate coefficients. With this differential equation, we couple the conditions $L(0) = S_p T$ since at the start of the experiment, we assume that any surface GLUT4 is instantaneously labelled. Also, eventually all GLUT4 in the trafficking cycle will be exposed to the surface at some point and thus become labelled (i.e. $\lim_{t\to\infty} L(t) = T$). The reader is encouraged to use separation of variables or the integrating factor technique to see that this system yields the solution (known as an analytic solution):

$$L(t) = T\left(1 - S_x e^{-k_{ex}t}\right). \tag{9}$$

We can approach the model transition experiment in a similar manner. However, this time the experiment is complicated by the fact that between the transition between the basal state and the insulin stimulated state, the trafficking rates, k_{ex} and k_{en}, are not constant. It is much more difficult to deal with differential equations with variable coefficients and so we must make further simplifying assumptions: we assume that the

cells are initially in a steady base state and upon insulin exposure, the rate constants instantaneously change to their insulin stimulated values. We can translate these assumptions mathematically: initial basal state implies that at $t = 0$, the concentrations of GLUT4 in each compartment are specified by (8) with $S_x = S_x^{basal}$ and $S_p = S_p^{basal}$ with rate constants $k_{ex} = k_{ex}^{basal}$ and $k_{en} = k_{en}^{basal}$. However, due to instantaneous change upon insulin exposure, all rate constants for $t > 0$ take on their insulin stimulated values ($k_{ex} = k_{ex}^{insulin}$ and $k_{en} = k_{en}^{insulin}$). Therefore, we have precisely the solution given by (7) with $p_0 = S_p^{basal} T$:

$$p(t) = S_p^{insulin} T + e^{-(k_{en}+k_{ex})t} \left[S_p^{basal} - S_p^{insulin} \right] T. \tag{10}$$

Now that we have the analytic descriptions, we can fit the solutions (9) and (10) to their data respectively. Here the parameter T (the total GLUT4 participating in the trafficking pathway) was allowed to vary. The comparison to the experimental data is shown in Figure 6. For the purposes of this study, MATLAB was used to perform the fits.

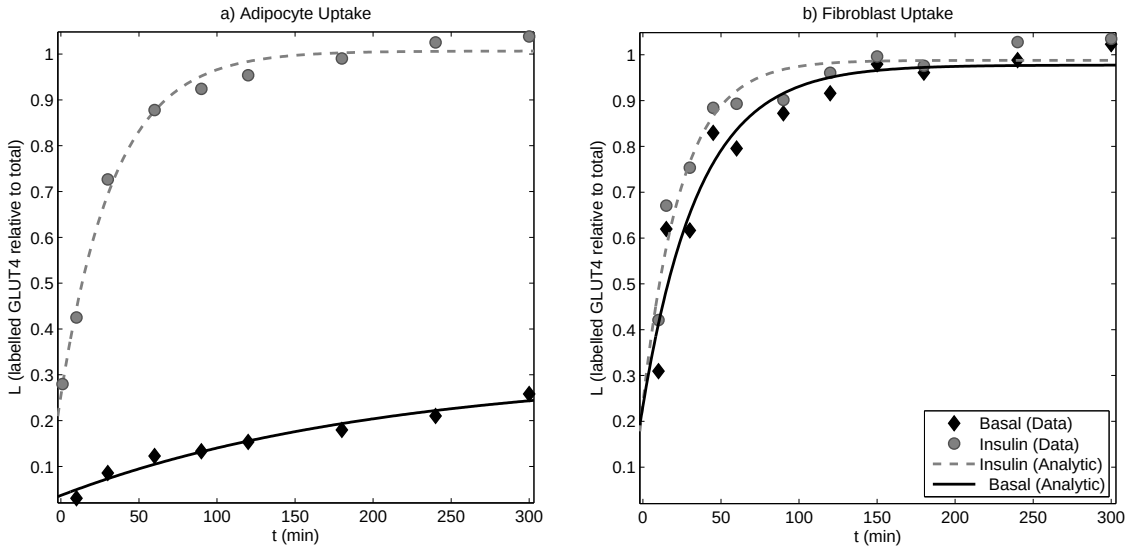

Figure 6: Two compartment model uptake experiment plot of L against t (min). (a) Fat cell (Adipocyte) approximate parameter estimates by curve fitting: base ($k_{en} = 0.036,\ k_{ex} = 0.005,\ T = 0.307$) and insulin ($k_{en} = 0.086,\ k_{ex} = 0.029,\ T = 1.007$). (b) Pre-fat cell (Fibroblast) parameter estimates: base ($k_{en} = 0.0882,\ k_{ex} = 0.0277,\ T = 0.978$) and insulin ($k_{en} = 0.125,\ k_{ex} = 0.043,\ T = 0.9879$). Data from supplied by Adelle Coster (School of Mathematics and Statistics, UNSW) and Cynthia Mastik (Department of Biochemistry and Molecular Biology, University of Nevada School of Medicine).

Throughout this investigation, we have illustrated the construction of compartment models. Our current model is now sufficiently sophisticated to qualitatively reflect

some behaviour of the system. However, it may not be the only model to do so. From here, we may begin to investigate more quantitatively (for example, by fitting our analytic solutions with biologically informed range of values to the data and arrive at rate constant estimates, goodness of fit and confidence bounds). We can compare such quantitative predictions to that of the literature on GLUT4 and glucose transport. This will allow us to further assess the appropriateness of our model. In fact, our current model will undoubtedly turn out to be insufficient and will require even further development. Regardless, each new development yields a more sophisticated model which will continue to inform and be informed by biological research.

Acknowledgments

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