SENSITIVITY ANALYSIS IN SYSTEMS BIOLOGY MODELLING AND ITS APPLICATION TO A MULTI-SCALE MODEL OF BLOOD GLUCOSE HOMEOSTASIS

Thomas Sumner

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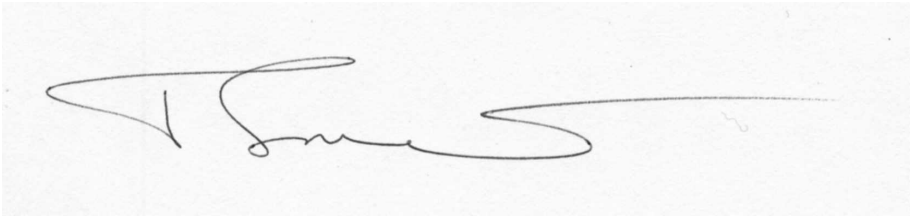
Centre for Mathematics and Physics in the Life Sciences and Experimental Biology

University College London

Declaration

I, Thomas Sumner, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.



Abstract

Biological systems typically consist of large numbers of interacting components and involve processes at a variety of spatial, temporal and biological scales. Systems biology aims to understand such systems by integrating information from all functional levels into a single cohesive model. Mathematical and computational modelling is a key part of the systems biology approach and can be used to produce composite models which describe systems across multiple scales. One of the major difficulties in constructing models of biological systems is the lack of precise parameter values which are often associated with a high degree of uncertainty. This uncertainty in parameter values can be incorporated into the modelling process using sensitivity analysis, the systematic investigation of the relationship between uncertain model inputs and the resulting variation in the model outputs.

This thesis discusses the use of global sensitivity analysis in systems biology modelling and addresses two main problem areas: the application of sensitivity analysis to time dependent model outputs and the analysis of multi-scale models. An approach to the analysis of time dependent model outputs which makes use of principal component analysis to extract the key modes of variation from the data, is presented. The analysis of multi-scale models is addressed using group-based sensitivity analysis which enables the identification of the most important sub-processes in the model. Together these methods provide a new methodology for sensitivity analysis in multi-scale systems biology modelling.

The methodology is applied to a composite model of blood glucose homeostasis that combines models of processes at the sub-cellular, cellular and organ level to describe the physiological system. The results of the analysis suggest three main points about the system: the mobilisation of calcium by glucagon plays a minor role in the regulation of glycogen metabolism; auto-regulation of hepatic glucose production by glucose is important in regulating blood glucose levels; time-delays between changes in blood glucose levels, the release of insulin by the pancreas and the effect of the hormone on hepatic glucose production are important in the possible onset of ultradian glucose oscillations. These results suggest possible directions for further study into the regulation of blood glucose.

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Chapter 1

Introduction

This chapter introduces the concept of systems biology and the use of mathematical models to study biological systems. The main issues associated with the construction of systems biology models are presented and the motivation and objectives for this research are discussed. The chapter concludes by outlining the structure of the rest of the thesis.

1.1 Systems Biology

Biological systems, from gene networks and intracellular signalling pathways to organs and complete organisms, consist of large numbers of components. The function and behaviour of such systems can only occasionally be understood by studying the parts of the system (genes, proteins, cells, organs) in isolation (Sauer et al., 2007). Rather, it is through the interactions of the components that the properties and functions of these systems emerge (Editorial, 2004). To understand the behaviour of such systems, we must not only study the component parts, we must also focus on understanding the structure and dynamics of the system.

The behaviour of a system at any given level of biological organisation is also dependent on the outputs and properties of systems at other levels. It is therefore important to consider the hierarchy of biological levels and the ways in which they interact (Editorial, 2004). This requires methods for simultaneously studying different levels of biological organisation (Dubitzky, 2006).

These are the approaches advocated in the field of systems biology (Kitano, 2002b). In contrast to a reductionist approach, in which components such as genes or proteins are studied one at a time, systems biology "seeks to understand complex biological systems in their entirety by integrating all levels of functional information into a cohesive model" (Thiel, 2006).

While the modern concept of systems biology is a relatively new field, there is a long history of systems thinking in biology. Bertalanffy (1968) emphasised the importance of a systems approach

in a variety of fields including biology in his concept of "systems theory" and classical physiology has long adopted a systems-level view (Kitano, 2002a). The current systems biology movement is characterised by its interdisciplinary nature involving the integration of experimental data from multiple sources with computational and mathematical models and techniques (Sauer et al., 2007).

1.1.1 Modelling

Mathematical and computational models are a major tool for understanding biological processes and a key part of the modern systems biology approach (Coatrieux, 2004). Modelling provides a method for formally defining and analysing the structure of a system and allows us to combine knowledge from different biological levels.

Mathematical models can be used for a number of purposes. They aid understanding by allowing us to compare competing hypotheses about the underlying mechanisms involved in a process. They may also suggest new hypotheses and experiments to test them. Models can also be used to analyse the system behaviour, in particular the response to external stimuli and perturbations. This can help locate important components of the system, investigate system robustness and identify weaknesses in the model. Models may also be used to help "design" aspects of biological systems to produce desired outputs (Bogle et al., 2009). In biotechnology or synthetic biology applications the aim may be to optimise the production of system components (Brent, 2004) while in physiology the aim is to design therapeutic interventions or treatments.

Model development typically follows an iterative cycle (Hangos and Cameron, 2001). Based on existing knowledge a model structure is proposed. Available data is then used to provide values for model parameters and initial conditions. The model is then validated against new data, often taken from the literature to minimise development time and costs (van Riel, 2006). The model is then refined based on the level of success of the validation stage. This process is usually repeated, incrementally improving the predictive power of the model against experimental observations.

Examples of computational modelling in systems biology range from simulations of intracellular signalling pathways (Lukas, 2004a,b) via models of whole cells (Nakayama et al., 2005) and complete organs (Noble, 2007) to the Physiome Project which aims to *"provide a framework for modelling the human body, using computational methods that incorporate biochemical, biophysical and anatomical information on cells, tissues and organs"* (Hunter and Borg, 2003). The work presented in this thesis is largely concerned with the latter, models which cross a variety of biological scales, combining information from sub-cellular, cellular and tissue levels to study physiological processes.

1.1.2 Challenges

Multi-scale systems biology modelling projects bring a number of challenges. Three key issues are discussed below: the difficulties of modelling across scales; the task of managing the data required for and generated by modelling projects; the selection or estimation of precise parameter values.

As discussed above, biological systems involve processes at a variety of spatial and temporal scales, and at different biological levels including intracellular networks, cell-cell interactions and organ structure. Models constructed at each level will use different modelling paradigms and employ varying degrees of simplification. The choices at each level will be motivated by a number of factors including the level of knowledge of the system, the availability of data and the computational demands of different approaches. The purpose or goal of the model should also be taken into consideration when making these decisions (Cameron et al., 2005). A major computational challenge is how to combine these models based on different algorithms, time-scales and levels of detail, to produce multi-scale models which will allow us to investigate the system level behaviour. Takahashi et al. (2004) suggest that there are two main approaches to solving this problem. The first is to develop a combined algorithm which "binds strengths of existing simulation algorithms to produce a unified simulation algorithm of wide utility". The alternative is to embed existing algorithms in some generic framework of "time advance and inter-module communication". The latter appears more fruitful. One example of this approach is the E-Cell Project (Matsuzaki, 2008), a modelling framework designed for the simulation of whole cells. There are also a number of general simulation frameworks, not specific to biological modelling, which are designed to allow the integration of models at multiple scales. These include the high level architecture (HLA) (Kuhl et al., 2002) and the dynamic information architecture system (DIAS) (Campbell and Hummel, 1998).

Another challenge is how to manage the mass of information associated with a modelling project. In addition to the model equations, this information includes details of the biology represented by the model, parameter values and their sources, version history and model outputs. The curation of models and the associated data is crucial to facilitate model reuse and the composition of larger models (Le Novere, 2006). To address this problem a number of repositories have been created for the storage and curation of published models including the BioModels database (Le Novere et al., 2006), the CellML model repository (Lloyd et al., 2008) and JWS Online (Olivier and Snoep, 2004). In addition a number of languages have been developed specifically for the representation of biological models. Such languages provide a common format in which to represent models, allowing them to be shared and reused by researchers working with a variety of software

tools. Two of the most successful are CellML (Lloyd et al., 2004) and the Systems Biology Markup Language (SBML) (Hucka et al., 2003), both of which are XML (Extensible Markup Language) based.

These two challenges were the focus of the UCL Beacon project "Vertical Integration Across Biological Scales" (Finkelstein et al., 2004), a collaborative effort to develop tools and methodologies to tackle organ modelling projects, which was undertaken between 2002 and 2007. The project developed a modular approach to model construction in which "composite" biological models are constructed by connecting together smaller "component" models of individual phenomena and processes. These component models may be constructed in different mathematical formalisms or languages and, where possible, the reuse of existing models taken from the published literature was recommended. A framework and model description language was developed which allows such composite models to be specified and executed (Margoninski et al., 2006). This is an example of the second approach to model construction outlined above. This framework was coupled with a model management system and database applications to capture and share the information associated with the models (Hetherington et al., 2006a).

One of the greatest challenges when building models of biological systems is estimating parameter values. The behaviour of the system may be strongly dependent on the values of some or all of the parameters so "accurate and reliable quantification" (van Riel, 2006) is necessary for the development of models. In reality the identification of exact parameters is a difficult task. Values for specific parameters, such as intracellular reaction rate constants, measured *in vivo* are rare (Zheng and Rundell, 2006) and it is more typical for parameters to be estimated from experimental measurements made *in vitro*. These may not accurately reflect the situation in the complete system. Different laboratories may report different values based on different techniques and conditions. Where parameter values can not be derived experimentally they may be estimated by fitting of model simulations to experimental data.

As a result, parameters are often estimated within large ranges or associated with a high degree of uncertainty. To deal with this "mismatch between available experimental data and modelling requirements" various approaches for dealing with "incomplete information" in biological modelling have been proposed (De Jong and Ropers, 2006). One approach is the use of sensitivity analysis (SA) to investigate the effects of the uncertainties in parameters on the model behaviour. SA is used in a variety of disciplines from environmental science to software engineering and in many fields is seen as "a prerequisite for model building" (Saltelli et al., 2000a). In addition to incorporating parameter uncertainty into the model, SA can be used to answer many of the questions typically addressed via biological models (see section 1.1.1). In particular, SA examines the response of

a model to perturbations, shedding light on the robustness of the model and helping to identify control points in the system.

1.2 Research Area

My research will focus on the use of SA in biological modelling. While there is a history of using SA in biology, in particular the use of metabolic control analysis (MCA), its application to multi-component or multi-scale models of physiological systems is limited. SA has many potential benefits in such cases:

- These models may contain large numbers of parameters whose values are uncertain or poorly constrained. SA allows this uncertainty to be incorporated into the modelling process and the resulting output uncertainty to be quantified.
- To reduce model output uncertainty, experimental effort should be focussed on refining those parameters which contribute most to the variation. SA can be used to determine those parameters and quantify their impact.
- SA can be used to identify the parts of the model which have no effect on system behaviour. These parts may be removed or simplified, reducing model complexity.
- The complex structure of such models means the effects of perturbing the system will not be obvious. SA provides a method for systematically investigating the effects of perturbations, identifying those parameters which drive system output and suggesting targets for interventions.

SA should be seen as a powerful tool for the construction and analysis of biological models. The development and application of appropriate methods is an important task in the continued success of an integrated approach to systems biology. This thesis will present the development of a number of techniques, which build on existing methods, and provide a methodology for performing sensitivity analysis of composite multi-scale biological models.

1.2.1 Context

The research described above will be carried out in the context of the UCL Beacon project (see section 1.1.2). The project, which developed an approach to the construction and management of systems biology models, focussed on the human liver and its role in glucose homeostasis as an example system.

Glucose is a major source of energy for the body in particular the brain and, as the brain cannot store or produce glucose it requires a regular supply from the circulation. The level of glucose in the blood must be tightly controlled (between 4.0-9.0mM) (Gerich, 2000) to maintain normal physiological function. Prolonged hypoglycemia, a reduced blood glucose level, can result in brain injury while hyperglycemia, elevated plasma glucose, leads to complications in the micro- and macrovascular system which can result in increased risk of cardiovascular disease (Reusch, 2003). The failure of the glucose regulatory system is also an integral part of several physiological disorders, most notably diabetes mellitus. It is estimated that the condition affects 171 million people worldwide, a figure which is predicted to rise to 366 million by 2030 (Wild et al., 2004).

Using the modelling framework developed during the project, a composite model of glycogen synthesis and breakdown in response to changes in the blood glucose level was produced. The component models of this system will be used as examples for the development of the SA techniques and the potential of the methodology will be demonstrated by application to the complete model.

1.3 Report Overview

Chapter 2 of this report presents a critical review of the published literature on the use of sensitivity analysis to deal with sources of uncertainty in biological modelling.

In chapter 3 I will give an overview of the modelling approach developed during the UCL Beacon Project and the glucose homeostasis model which was produced. This chapter will also discuss my development of a more mechanistic model of the insulin signalling pathway, a key part of the glucose regulatory system. These models are used as examples in my research into sensitivity analysis techniques.

Chapters 4 and 5 describe the development of SA techniques which address various issues related to the analysis of multi-scale systems biology models.

Chapter 6 presents a case study in which the methods are applied to the composite model of glucose homeostasis.

Finally, in chapter 7 the conclusions of the research are presented and possible directions for future work are discussed.

Chapter 2

Applications of Sensitivity Analysis in Systems Biology

This chapter presents a critical review of the published literature on the use of sensitivity analysis in biological modelling. The concept of sensitivity analysis is introduced and the reasons for its use in biological modelling are stated. The various sensitivity analysis approaches found in the biological literature are then presented. The chapter concludes by highlighting the areas where additional research is required and stating the aims of this thesis.

2.1 Introduction

The term sensitivity analysis (SA) has a variety of meanings in different disciplines. A good general definition was given by Nestorov (1999) who described SA as "the systematic investigation of the model responses to either i) perturbations of the model quantitative factors (e.g. inputs and/or parameters) or ii) variations in the model qualitative factors (e.g. structure, connectivity modules or submodels)".

The majority of work in the field of SA has focussed on the investigation of quantitative factors. Complex mathematical and computational models typically contain large numbers of parameters whose values are not precisely known. Uncertainty in those values produces uncertainty in the output of the model. Understanding and quantifying this uncertainty using sensitivity analysis is an important part of the development and use of models (Saltelli et al., 2000b).

There are two main classes of SA: local methods, in which inputs are varied one at a time by a small amount around some fixed point and the effect of individual perturbations on the output are calculated; global methods, in which all inputs are varied simultaneously over their entire input

space, typically using a sampling based approach, and the effects on the output of both individual inputs and interactions between inputs are assessed. The use of both classes to study the sensitivity of quantitative input factors in biological models will be discussed in this chapter.

2.1.1 Sensitivity Analysis in Biological Modelling

The estimation of precise parameter values is a major issue in the construction of biological models. The model behaviour may be strongly dependent on the parameters (van Riel, 2006) and if those parameters are uncertain any conclusions drawn from the model output must take into account that uncertainty. This lack of precise parameter values can be addressed using sensitivity analysis (De Jong and Ropers, 2006). By incorporating the uncertainty in parameters into the model we can quantify the uncertainty in the output and in inferences we make from it.

Sensitivity analysis also allows us to analyse the affects of perturbations of the system from its normal state and identify the parameters which are important in controlling the system behaviour. This information can be useful in both an "understanding" context, suggesting hypotheses about important mechanisms in a system, and a "design" context, suggesting how we may intervene in the system to produce certain behaviours.

The use of sensitivity analysis is well established in mathematical modelling in many fields including biology (Hetherington et al., 2006b). The best known example of SA in biology is the use of metabolic control analysis (MCA) in the study of metabolism. The use of SA in other areas of biology, such as cellular signalling, is less common (Hu and Yuan, 2006) although there are a growing number of examples. Applications to multi-scale biological models are rare. The rest of this chapter will discuss the use of SA in biology making reference to more general literature where appropriate.

2.2 Metabolic Control Analysis

MCA was developed to "elucidate in quantitative terms to what extent the various reactions of metabolic pathways determine the resulting fluxes and metabolite concentrations" (Heinrich and Schuster, 1996). The basis of MCA are the various forms of control coefficient which measure the response of the system variables after parameter perturbations. An example is given by the flux control coefficients, defined as:

$$C_{v_k}^{J_j} = \left(\frac{v_k}{J_j} \frac{\Delta J_j}{\Delta v_k} \right)_{\Delta v_k \to 0} = \frac{v_k}{J_j} \frac{\partial J_j}{\partial v_k} = \frac{v_k}{J_j} \frac{\partial J_j / \partial p_k}{\partial v_k / \partial p_k} \tag{2.1}$$

where J_j is the steady state flux of metabolite j and Δv_k is the change in the activity of a reaction k due to a change in a single parameter p_k.

Similar equations can be specified for the control coefficients of the steady state concentrations and a number of other coefficients have been proposed. A detailed description of MCA and its applications can be found in (Heinrich and Schuster, 1996). In the early work of Kacser and Burns (1973) control coefficients were referred to as sensitivities, highlighting the fact that MCA is a specific example of the more general approach of local sensitivity analysis.

In the majority of MCA applications, the sensitivities are calculated at steady-state (Hu and Yuan, 2006). In many systems, such as signal transduction pathways, it is the transient behaviour of the system which is of more interest. MCA in its original form is not well suited to the study of such processes (Liu et al., 2005). Ingalls and Sauro (2003) extended many of the concepts of MCA to dynamical systems by defining time-varying concentration sensitivity coefficients which measure the response to a perturbation along the entire model output trajectory. These coefficients are equivalent to the time-dependent sensitivities defined in local sensitivity analysis and discussed in the following section.

2.3 Local Sensitivity Analysis

For a general ODE model of the form:

$$\frac{d\mathbf{y}}{dt} = f(\mathbf{y}, \mathbf{k}), \quad \mathbf{y}(0) = \mathbf{y}^0 \tag{2.2}$$

where $\mathbf{y}$ is the vector of variables, $\mathbf{k}$ is the m-vector of system parameters and $\mathbf{y}^0$ are the initial values, the effect of a small parameter change on the solution can be expressed as a Taylor series expansion:

$$y_i(t, \mathbf{k} + \mathbf{\Delta k}) = y_i(t, \mathbf{k}) + \sum_{j=1}^{m} \frac{\partial y_i}{\partial k_j} \Delta k_j + \frac{1}{2} \sum_{l=1}^{m} \sum_{j=1}^{m} \frac{\partial^2 y_i}{\partial k_l \partial k_j} \Delta k_l \Delta k_j + ... \tag{2.3}$$

The partial derivatives $\partial y_i / \partial k_j$ are known as the first-order local sensitivity coefficients and form the sensitivity matrix $\mathbf{S}(t) = \{s_{ij}\} = \{\partial y_i / \partial k_j\}$. $s_{ij}(t)$ describes the effect on the i^{th}output variable at time t of a small change in the j^{th} parameter around its nominal value. Generally it will not be possible to find an analytical solution so numerical methods must be used to calculate $\mathbf{S}$ at each time point.

2.3.1 The Indirect Method

The "simplest conceptual route to calculating the local sensitivities" (Rabitz et al., 1983) is the indirect or finite-difference method. Using this method the model is solved at some chosen parameter point and then at some perturbed value of each parameter, $k_j + \Delta k_j$ while all other parameters are held at their nominal values. The sensitivities can then be calculated using a forward difference approximation.

$$s_{ij}(t) \approx \frac{y_i(k_j + \Delta k_j, t) - y_i(k_j, t)}{\Delta k_j} \tag{2.4}$$

The indirect method requires at least $m+1$ runs of the model (this rises to $2m$ if central differences are used). For models with large numbers of parameters, or those that have significant run-times this can make the indirect method computationally intensive.

Perhaps the biggest challenge when using the indirect approach is the selection of the parameter step size. The finite difference approximation assumes local linearity around the nominal parameter point. If the step size is too large this assumption does not hold. Conversely, if the step size is too small, the difference between the original and perturbed solutions can be so small that numerical errors in the solution become an issue. Saltelli et al. (2000a) state that finding the best value is a trial and error process. De Pauw and Vanrolleghem (2003) assessed the "quality" of the resulting sensitivity coefficients as the step size was changed. Their results indicated that the optimum step size was both parameter and variable specific and as such could not be easily generalised.

Despite its problems, and recommendations against its use (Turanyi, 1990), the indirect approach is still frequently used. The primary reason is due to its simplicity and that, unlike the direct approaches to be discussed below, it requires no extra "numerical machinery" (Rabitz et al., 1983) other than that needed to solve the system of ODEs. More sophisticated methods require access to and modification of the model code, something which is not always possible or desirable (De Pauw and Vanrolleghem, 2003).

2.3.2 The Direct Method

In the direct approach the model equations (2.2) are differentiated with respect to the parameter k_j to give the following system of sensitivity differential equations:

$$\frac{d}{dt}\frac{\partial \mathbf{y}}{\partial k_j} = \mathbf{J}(t)\frac{\partial \mathbf{y}}{\partial k_j} + \frac{\partial \mathbf{f}(t)}{\partial k_j} \tag{2.5}$$

where $\mathbf{J}(\mathbf{t}) = \partial\mathbf{f}/\partial\mathbf{y}$ and the initial condition for $\partial\mathbf{y}/\partial k_j$ is a zero vector.

There are a number of efficient methods to solve the sensitivity equations the most general of which is the decoupled direct method (DDM) (Saltelli et al., 2000a). The direct method has become increasingly popular in biology and has been applied in the analysis of a number of signal transduction pathways. Yue et al. (2006) used the DDM to perform local sensitivity analysis of a model of the NF-κB signalling pathway to identify the parameters which had an influence on the oscillatory behaviour of the system. A similar approach was used by Hu and Yuan (2006) to study the coupled MAPK-PI3K pathways and identify the most sensitive reaction steps. Liu et al. (2005) also used the DDM to calculate the sensitivity of species concentrations in the epidermal growth factor (EGF) mediated signalling network to changes in reaction rates as a function of both time and EGF stimulus dose. This study highlighted the fact that in addition to varying with time, sensitivities can be dependent on the external input to the model: the system was found to be increasingly sensitive to internalisation processes at lower stimulus doses.

2.3.3 Feature Sensitivity Analysis

Turányi and Rabitz (in Saltelli et al., 2000a, chp. 5) suggest that in many cases we should be more interested in the sensitivity of aspects of the model output rather than the sensitivity of the output at a given time point. This is likely to be the case in models of biological systems where we may wish to answer questions such as, what influences the maximum value of the species concentration or how does the period of an oscillatory solution vary with the model parameters?

Frenklach (1984) suggested that the indirect method could easily be used to calculate "feature" sensitivities by evaluating the feature from the original and perturbed model solutions and using finite differences to find the sensitivities. As with the standard indirect method this approach is very easy to implement and has been used in several studies of biological systems (Ihekwaba et al., 2004; Hetherington et al., 2006b). The main problem with this approach is that it is very model specific and its application is somewhat ad-hoc. For any given model we must select suitable features and ideally implement computational algorithms to evaluate them. In some cases a feature may not be present in all model runs (for example only certain parameter values may generate oscillations in the model output). Even if the feature does exist it is possible that any automated procedure may not locate it. This problem was encountered by Ihekwaba et al. (2005) in their analysis of the NF-κB signalling pathway. They simply chose to ignore the missing values.

Feature sensitivities can also be derived from so called "elementary sensitivities" calculated via the direct method. Goldenberg and Frenklach (1995) suggest the following procedure. The solution to the model at parameter point $\mathbf{k}$ can be expanded into a Taylor series at each time

point with respect to the parameter of interest k_j. Truncating the expansion after two terms, the perturbed solution can be approximated as:

$$\tilde{\mathbf{y}_j} = \mathbf{y} + \mathbf{S}_j \Delta k_j \tag{2.6}$$

where $\mathbf{S}_j$ are the sensitivities of the output to parameter k_j. The feature of interest can now be evaluated from the original and approximated perturbed solution and its sensitivity to k_j calculated as:

$$S_{F,j} = \frac{\tilde{F}_j - F}{\Delta k_j} \tag{2.7}$$

The authors found that this approximate approach produced results in good agreement with the indirect approach discussed above while avoiding the need for numerous runs of the model. However it does not overcome the other issues with the indirect method. It is still necessary to make a suitable choice for Δk_j. Nor is it any less model specific than the use of the indirect method. The features must still be selected and evaluated from the original solution and the approximated perturbed output.

2.3.4 Limitations of Local SA

Local sensitivity analysis techniques have been applied in a number of signal transduction and metabolic pathway models to analyse the time-dependent behaviour and identify important parameters and reaction steps. However local methods have a number of limitations. Firstly they only investigate the behaviour of a model in the immediate region around the nominal parameter values. In biology, input values are often very uncertain and cover large ranges which can not be investigated using local techniques (Marino et al., 2008). Secondly, local techniques only consider changes to one parameter at a time, with all other parameters fixed to their nominal values. In biological systems it is likely that interactions between parameters will be important. Therefore it is necessary to investigate the effects of simultaneous parameter variations of arbitrary magnitude (van Riel, 2006). This requires the use of global SA methods.

2.4 Global Sensitivity Analysis

It is only relatively recently that global SA techniques have begun to be applied to biological models (van Riel, 2006). In this section we discuss the application of a number of global methods to models of biological systems.

2.4.1 Sampling Based Methods

Sampling-based methods use Monte-Carlo (MC) techniques to explore the mapping between uncertain model inputs and outputs. For a model with k inputs $\mathbf{x} = [x_1, x_2, ..., x_k]$ a general sampling-based approach involves five main steps (Saltelli et al., 2000a):

1. Define distributions $D_1, D_2, ..., D_k$ that characterise the uncertainties in the inputs $\mathbf{x}$
2. Generate a sample of size N, $\mathbf{x}_1, \mathbf{x}_2, ..., \mathbf{x}_N$, from the distributions defined in step 1
3. Evaluate the model for each element in the input sample to obtain a set of model outputs, $\mathbf{y}(\mathbf{x}_i), i = 1, 2, ..., N$
4. Quantify and display the uncertainty in the model outputs
5. Explore the mapping between uncertain inputs and the output uncertainty

The output of any MC analysis is very sensitive to the input distributions (Lipton et al., 1995) therefore the characterisation of those distributions is probably the most important part of sampling-based methods (Saltelli et al., 2000a). The choice of distribution will depend on the purpose of the analysis and the available knowledge on the parameter values. When sufficient information is available this can be used to assign specific distributions for each parameter, either via parametric fitting to known distributions or using non-parametric density estimation techniques (Silverman, 1986). For initial explorations of a model or when there is limited data on the weighting of particular parameter values it may only be possible to identify minimum and maximum values of a parameter. The natural choice is then to assume a uniform distribution across this range (Lipton et al., 1995). This lack of information is often encountered in biological modelling and uniform distributions are typically used. This is the approach taken by Segovia-Juarez et al. (2004) in their analysis of a model of granuloma formation during *M. tuberculosis* infection.

The simplest way to generate a sample from the input distributions is to use random sampling. The main issue with random sampling is that a large number of samples may be required to ensure that the entire range of each input is sampled appropriately (Saltelli et al., 2000a). If the model of interest is expensive to evaluate this can be a problem. Latin hypercube sampling (LHS) (McKay et al., 1979) is a sampling procedure which has been shown to be more efficient than random sampling (Helton and Davis, 2003). In LHS, the range of each input is divided into n_{LHS} intervals of equal probability. One value is selected at random from each interval for each input and the values combined in a random manner without replacement to produce n_{LHS} samples. LHS ensures the entire range of each input is sampled and has been used in the analysis of a number of biological

systems (Segovia-Juarez et al., 2004; Marino et al., 2008). An alternative to LHS are quasi-random sequences such as the Sobol sequence which will be discussed below in relation to variance based SA methods.

Once the input samples have been generated the third step is to evaluate the model for each set of inputs and to store the results of each run. The details of this step are model and application (the programme or language used to run the model) specific.

Uncertainty analysis of the model outputs can be performed in many ways. The first step is to assess the overall uncertainty in the model output. For scalar model outputs this can be summarised by the mean value and variance. More information can be obtained by plotting the probability density function (PDF) or cumulative distribution function (CDF) of the output. If the model output is time dependent, Helton and Davis (in Saltelli et al., 2000a, chp. 6) suggest plotting the point-wise mean together with some appropriate point-wise percentiles to obtain a picture of the output uncertainty.

The final step is to explore the effects of individual parameters on the model outputs. The simplest approach is to examine scatter plots of the model output against parameter values for each parameter. This approach is not practical for use with time-varying model outputs as we would need to generate and examine a large number of plots, one for each time-point of interest. A more quantitative assessment can be performed using regression or correlation analysis (Helton and Davis, 2003). Several authors have made use of partial rank correlation coefficients (PRCC) to study biological systems including Blower and Dowlatabadi (1994) who utilised SA to investigate a model of HIV transmission and Segovia-Juarez et al. (2004) (see above). Such measures may be calculated for scalar model outputs or at multiple time-points to investigate the sensitivity of dynamic model outputs.

The problem with regression and correlation based indices is that they are only suitable when the relationships between the parameters and the model output satisfy certain conditions of linearity or monotonicity. Marino et al. (2008) applied various SA techniques to a number of biological models and demonstrated that PRCCs are not accurate when non-monotonicities are present. As there is no way to know a priori whether or not these conditions are satisfied they suggest it is necessary to utilise methods which have no such constraints.

2.4.2 Variance Based Methods

Unlike the various forms of regression or correlation measures, variance based methods are model-free, they are not dependent on assumptions about the relationships between model inputs and outputs (Saltelli et al., 2000a). These methods are based on a partitioning of the total output

variance and identify the amount of variation which is explained by the uncertainty in the parameters. Variance based measures are very powerful in "quantifying the relative importance of input factors" (Saltelli et al., 2004). In addition to considering the importance of individual inputs (their "main effects") variance based methods can also be use to investigate the effects of interactions between parameters. Usefully, this allows the "total effect" of a parameter, which includes all its possible interactions with other parameters, to be quantified. As with PRCCs (and other forms of correlation based measures) the variance based methods can be applied to scalar outputs or to time-varying model outputs in a point-wise manner.

Two main approaches are commonly used for the calculation of the variance based sensitivity indices. The Fourier amplitude sensitivity test (FAST) (Cukier et al., 1978) and its extended version (eFAST) (Saltelli et al., 1999) (developed to allow the computation of "total effect indices") are based on an exploration of the uncertain parameters in the frequency space. eFAST was previously considered the most efficient way to compute the main and total effects and was used by Marino et al. (2008) as part of their methodology for applying global SA in systems biology. They suggested that variance based techniques are a key tool due to their model-independence.

An alternative variance based approach is the method of Sobol (Sobol, 1993) which is based on a decomposition of the variance into terms of increasing dimensionality. These partial variances are estimated using MC integrals and the sensitivities are based on their ratio to the total variance. The Sobol method is an attractive approach to the calculation of variance based indices as it is relatively easy to implement. An improvement to the algorithm for computing the integrals (Saltelli, 2002) also improved the efficiency of the method, making it comparable to that of eFAST. The modified Sobol method requires $N(k+2)$ model evaluations to calculate one estimate of both the main and total effects, where N is of the order of a few thousand. The MC integral estimates converge to their true value as the sample size, N, is increased however there is no a priori way of knowing what N should be. In many applications this number can be reduced by using more efficient sampling strategies. Both LHS (see above) and quasi-random sequences have been used. Quasi-random sequences, such as the Sobol sequence, are deterministic sequences which maximise coverage of the multi-dimensional input space for a given sample size. These have been shown to be the most efficient sampling strategy under certain circumstances (Niederreiter, 1992) but their performance declines as the number of parameters (and hence the dimension of the input space) increases (Kucherenko et al., 2009). Zheng and Rundell (2006) calculated variance based measures using both the eFAST and Sobol methods in their comparative study of SA techniques applied to a model of the Erk-MAPK signalling pathway. Both methods were shown to produce consistent results for both main and total effects with a comparable computational cost.

Despite the improvements to efficiency of both the eFAST and Sobol methods variance based techniques can still be prohibitively time consuming if the model contains a large number of inputs or the model has a significant run time. In these circumstances an alternative approach is required.

2.4.3 Screening Methods

Screening methods are a class of sensitivity analysis techniques designed for use with models containing large numbers of input factors. Their defining characteristic is their economy: they typically require far fewer runs than alternative methods. The drawback to screening designs is that they only provide a qualitative measure of importance. Using these methods, parameters are ranked in order of importance but the difference in importance is not quantified. A number of screening designs have been proposed in the literature of which the most robust and effective is the Morris method (Morris, 1991; Campolongo et al., 2007). The Morris method uses the average and standard deviation of a number of local sensitivity measures (or "elementary effects"), evaluated at various points in the input space, to provide an approximate global importance measure. A high average value implies that a parameter is important, a high standard deviation implies that its effects are non-linear or the result of interactions with other inputs. The key to the Morris method is an efficient design for the selection of the input points which optimises coverage of the space and minimises the number of model evaluations required to calculate the elementary effects. This approach has been shown to produce good agreement with the Sobol method, identifying the same inputs as influential (Campolongo and Saltelli, 1997).

Due to its low computational cost the Morris method is an appropriate tool to study complicated biological system models involving large numbers of parameters. Jin et al. (2008) used the Morris method to study a model of circadian rhythm in *Neurospora*, a type of mould. The method was selected for its low computational cost in comparison with other global SA techniques. Yue et al. (2008) also used the method to study the NF-κB pathway which had previously been investigated using local methods (Ihekwaba et al., 2004, 2005; Yue et al., 2006). The global nature of the Morris method identified additional important parameters whose interaction effects were not captured by local SA.

Weighted Local Measures

A variation on the concept of the Morris method has been developed in the biological literature. Bentele et al. (2004), in their work on apoptosis, attempted to overcome the limitations of local analysis by calculating local measures at a number of random points in the input space. A weighted average of these local sensitivities was used to provide an approximation to the global importance

of each parameter. This method was compared to PRCCs and the variance based measures by Zheng and Rundell (2006) and found to be produce results which were inconsistent with the other global approaches. They suggested that the agreement between methods could be improved by increasing the sample size. This method appears to provide no benefit over the more established Morris method.

2.5 Regionalized Sensitivity Analysis

An alternative approach to the global sensitivity analysis methods discussed above, commonly termed regionalized sensitivity analysis (RSA), was introduced by Hornberger and Spear (Hornberger and Spear, 1980; Spear and Hornberger, 1980) in their model based analysis of estuarine eutrophication (the acceleration of the natural ageing of a body of water) in Western Australia. The key to RSA is the definition of the "behaviour" the model should reproduce. This is typically defined via a set of constraints, often specified as inequalities, against which the output of the model can be compared. The model is evaluated at various parameter values, using some form of sampling based method, and the resulting model outputs are classified as either satisfying (B) or not satisfying ($\overline{B}$) the defined behaviour. The distributions of individual parameters associated with B and $\overline{B}$ are then compared, in the original example using the Kolomogorov-Smirnov two sample test, to identify those parameters which are influential in determining whether or not the model produces the desired behaviour.

Unlike the methods discussed in the proceeding sections RSA incorporates the expected or desired behaviour of the real system into the sensitivity analysis procedure. While other global SA techniques identify the parameters which most influence the model output, RSA identifies the parameters which are most important in producing specific behaviours in the model. These may be qualitative, for example the presence of oscillations, or quantitative, the maintenance of a system output within certain bounds. This may be useful in systems biology, particularly if we are interested in designing interventions to produce specific behaviour in the system.

The RSA approach has been applied in a biological context by Cho et al. (2003) and Zi et al. (2005). They called the method multi-parametric sensitivity analysis (MPSA) and used it to identify the key components in the NF-κB and JAK-STAT signalling pathways respectively. In both studies a model run was classified as satisfying the desired behaviour if its deviation from the nominal model output was less than some threshold value. This form of "behaviour" definition does not fully exploit the potential of RSA to include the observed behaviour of the system in the analysis.

While RSA has global properties (parameters are varied simultaneously and over their entire ranges) it does not allow any investigation of interaction effects, as measured by the total effects of the variance based methods or the standard deviation of the Morris method. Due to this limitation Saltelli et al. (2004) suggest that further inspection of the unimportant factors is necessary to ensure they are not involved in higher order interaction effects. Saltelli et al. (2004) also highlight another limitation of RSA: it only considers variation in the acceptable-unacceptable direction so important parameters may be missed if they only cause variation within the behavioural class.

2.6 Cross Scale Sensitivity Analysis

The majority of applications of sensitivity analysis in the biological literature have focussed on a single level of biological organisation, typically sub-cellular signalling pathways. As discussed in chapter 1 the behaviour of biological systems are dependent on the interactions between different levels of organisation. Examples of sensitivity analysis of multi-scale models, investigating the effect of parameter uncertainties across scales, are rare.

In a recent paper Wang et al. (2008) discussed the concept of "cross-scale sensitivity analysis". They studied a model of non-small cell lung cancer in which an ODE model of the EGFR-ERK signalling pathway was coupled with a discrete 2-d lattice model describing the migration and proliferation of cells. At each timestep the phenotypic trait of each cell is determined by the outputs of its own sub-cellular pathway model. The study used an indirect local sensitivity analysis to investigate the effects of perturbations in the parameters of the signalling pathway model on the tumour expansion rate, a multicellular level output.

Marino et al. (2008) have also discussed the concept of multi-scale or multi-compartmental sensitivity analysis. They defined the terms intra-scale/compartment and inter-scale/compartment to describe parameters which affect outputs of the same or different scales/compartments respectively. These ideas were demonstrated on a model of tuberculosis infection which consisted of two compartments representing the lymph node and the lung. PRCCs and the eFAST method were used to identify both intra and inter-compartmental important parameters.

An alternative approach to the analysis of multi-compartment models can be found in the field of pharmacokinetic modelling. Nestorov (1999) introduced the concepts of auto and cross-sensitivity to analyse whole body physiologically-based pharmacokinetic (PBPK) models. PBPK models are used to study the absorption, distribution, metabolism and excretion of compounds in humans and other animal species and consist of multiple compartments representing the various tissues of the body. The effect of perturbing a parameter in a given tissue compartment was factorised into

the resulting perturbation of the compound concentration in that tissue (auto-sensitivity) and the effect of this tissue level change on the response of all other tissues (cross-sensitivity). This concept has potential in systems biology modelling where a similar division could be made between, for example, the effect of a rate constant on the output of its signalling pathway and the effect of a perturbation in that output on the cellular or tissue level. Such a division could be used to investigate the role of sub-processes on the system response. However, the method proposed by Nestorov (1999) for calculating the sensitivities was based on a local approach and specific to the form of PBPK models. This makes it unsuitable for use in a more general modelling context.

2.7 Conclusions

This chapter has presented a review of the published literature on the use of sensitivity analysis in biological modelling. The concept of sensitivity analysis has a long history in biology in the form of MCA. More recently the potential benefits in a wider setting have been recognised. There are a growing number of applications of SA to be found in the literature, applied to a variety of systems and using a range of techniques.

From the literature review three main issues surrounding the use of SA in biological modelling can be highlighted. Firstly, there has been a reliance on local techniques in the biological literature. The limitations of these methods has been recognised and there has been a growth in the use of global techniques which should be continued. Secondly, in biology it is often necessary to study the sensitivity of dynamic model outputs and while methods exist for the analysis of such systems they have drawbacks. Finally, examples of the application of SA to multi-scale biological models are limited. This is an area in which the systematic approach of sensitivity analysis could be particularly useful. These three issues are discussed in more detail below.

2.7.1 Classes of SA

Sensitivity analysis techniques are typically divided into two broad classes. Local techniques, which address small scale perturbations of individual parameters around some fixed point and global techniques which investigate the simultaneous variation of model inputs over larger but finite regions. Examples of both local and global sensitivity analysis can be found in the biological literature. Until recently local methods were most common however more recently there has been a growth in the use of global methods. These are typically more appropriate for biological models where parameters may be associated with significant uncertainties and the likelihood of non-linear relationships and interactions between inputs is high.

Variance based techniques, such as the method of Sobol, are typically regarded as the most powerful and generally applicable form of global SA. They are model independent and are able to deal with both individual and interaction effects. Their utility in biological modelling has been demonstrated and their use merits further investigation. The main issue with the variance based techniques is their computational cost. Where this cost is prohibitive to timely analysis of the model screening designs have the potential to provide useful information on the model input/output relationships. One screening design in particular, the Morris method, has received increased attention in recent years and has been applied in a small number of biological modelling studies. The combined use of variance based techniques, where computational and time demands allow, and the Morris method, where they do not, would seem to represent a suitable approach in systems biology modelling. This approach will be used throughout this research.

2.7.2 Sensitivity Analysis of Dynamic Model Output

As discussed in section 2.3 in many systems it is the sensitivity of the transient or dynamic behaviour of the system which is of interest. The most straightforward approach to the local sensitivity analysis of such systems is to calculate time-varying sensitivities along the output trajectory (Ingalls and Sauro, 2003). An alternative method is to define a set of scalar values which describe the key features of the model output, for example the maximum value or the period of oscillations. Both of these approaches have been utilised in biological modelling but both have their drawbacks. By looking at individual time-points, it is possible we may miss interesting features of the model output. On the other hand, selecting a set of features is a highly problem-specific approach (Campbell et al., 2006). There will be many possible features to choose from and for any given model it is necessary to have some previous knowledge of the form of the output to make appropriate choices. The same methods are also used in the application of global techniques to dynamic models with the same drawbacks. There is the potential to develop alternative methods which overcome some of these issues and this will be one focus of this thesis.

2.7.3 Sensitivity Analysis of Multi-Scale Models

The use of SA has been largely limited to models which focus on single biological scales. Given the importance of hierarchical interactions in the function of most biological systems the development and analysis of multi-scale models is an important goal. Multi-scale models will often have complex structures in which the effects of uncertainties and perturbations will not be obvious. In addition they may include large numbers of uncertain parameters. The potential of sensitivity analysis in

such cases is clear.

One approach to the construction of multi-scale models is a modular approach in which models representing different aspects of the overall system are combined to produce a composite model. Sensitivity analysis techniques which make use of this modularity to investigate the importance of both individual parameters and entire sub-processes on the model behaviour would represent an advance on the current approaches to multi-scale SA.

2.7.4 Aims

The main aims of this thesis are summarised below:

- To develop a new approach for the global sensitivity analysis of dynamic model output
- To develop methods for the global analysis of multi-scale biological models
- To demonstrate the methods by application to a composite biological model

Chapters 4 and 5 discuss my development of sensitivity analysis methods which address the first two aims. Chapter 6 demonstrates the application of these methods via a case study of a composite model of blood glucose homeostasis which was developed as part of the UCL Beacon project.

The next chapter presents an overview of the glucose homeostasis model and the system it describes. It also discusses my development of a mechanistic model of the insulin signalling pathway, a key part of the regulatory system. This development was undertaken to address the lack of biological detail in that component model. The development highlights both the modular nature of the composite model and the reuse of published models.

Chapter 3

Multi-Scale Modelling of Blood Glucose Homeostasis

This chapter describes the composite multi-scale model of glucose homeostasis created at UCL and further developed during my research. It begins with a brief overview of the biological system, with particular reference to the role of the liver. The original component models which make up the composite model are then introduced. The second half of the chapter details my development of an alternative, more mechanistic, model of the insulin signalling pathway, a key component of the system.

3.1 Introduction

The UCL Beacon project (see chapter 1) was an interdisciplinary project focussing on the development of methods for the construction and management of multi-scale models of biological systems. During the project a modular approach to model construction was adopted. This approach advocated the construction of multi-scale models by connecting together smaller component models of phenomena and processes at different scales to produce a composite model of a system. This method facilitates the reuse of existing models and allows component models to be modified or replaced as and when new information about the system becomes available.

As an example of the approach adopted by the project a model of the glucose homeostasis system was produced, with the main emphasis on the role of the liver and in particular the processes of glycogen synthesis and breakdown. The model consisted of seven component models, some developed in-house, others taken from the published literature, which describe various aspects of

the biology. The models are connected via their inputs and outputs to produce a composite model which reproduces the system level behaviour, the regulation of blood glucose levels in response to external supply or demand. The composite model and its component sub-models provide an example system for the development and demonstration of the sensitivity analysis methodology presented in this thesis. This chapter provides an overview of both the biological system and the existing model.

The sub-models of the glucose homeostasis model are constructed at a variety of levels of detail depending on the existence of published models or the availability of experimental data and biological knowledge. While a simple model may accurately reproduce the observed behaviour of the sub-system the lack of detail limits its potential use for understanding and analysing the system behaviour. If a component or mechanism is not represented in a model it will not be possible to investigate its role using sensitivity analysis techniques.

This issue is particularly evident in the original model of the response of hepatocytes to insulin. The insulin signalling pathway is a key component in the regulation of glucose. Defects in this pathway can result in a reduced response of cells to insulin leading to insulin resistance which is the primary cause of type 2 diabetes. This form of the condition accounts for 90% of diabetes cases globally (Zimmet et al., 2001). Understanding the mechanisms underlying insulin resistance can aid efforts to develop new treatments for the disease (Brady and Saltiel, 1999).

I have addressed the lack of detail in the insulin component model by developing a mechanistic model of the insulin signalling pathway which is described in the second half of this chapter. The new model is a modification of the model of Sedaghat et al. (2002) and illustrates the benefits of model reuse in multi-scale systems biology modelling. The detailed model will allow us to investigate the potential effects of perturbations in the insulin pathway on the function of the glucose regulatory system.

3.2 Glucose Homeostasis

The regulation of blood glucose involves a balance between the supply of exogenous glucose from food and the demands of the body for energy. This balance is maintained by the storage of excess glucose (in the form of the polymer glycogen), its subsequent release, and the endogenous production of glucose from amino acid precursors (gluconeogenesis). The liver acts as a reservoir for excess glucose, storing glycogen for future use by other tissues. Following a mixed meal Taylor et al. (1996) estimate that $\sim 19\%$ of the ingested glucose is taken up by the liver and converted to glycogen. Similarly, the liver makes a major contribution to the postabsorptive (fasting) blood

glucose level being responsible for $\sim 80\%$ of glucose released into the circulation (Gerich, 2000). In the first 24 hours of fasting hepatic glycogenolysis accounts for between 40 and 80% of this glucose production (Bollen et al., 1998). During prolonged starvation gluconeogenesis begins to play an increasingly important role and is responsible for $93 \pm 2\%$ of glucose release after 42 hours (Landau et al., 1996).

3.2.1 Glycogen Metabolism

Glycogen synthesis and glycogenolysis (the breakdown of glycogen) are governed by the enzymes glycogen synthase (GSyn) and glycogen phosphorylase (GPho). GSyn produces glycogen by forming chains of glucose units linked via α-1,4 bonds. These chains are then combined (by branching enzyme) to form "bush like" glycogen particles. Glycogenolysis involves the removal of the branches by debranching enzyme and the liberation of individual glucose units from the free end of the chain by GPho (Bollen et al., 1998).

GSyn and GPho both have an active, a, and an inactive, b, form. GSyn is inactivated ($a \rightarrow b$) by the reversible phosphorylation of multiple serine residues by a number of protein kinases, including glycogen synthase kinase (GSK3) (Patel et al., 2004), and converted back to its active form by a protein phosphatase (PP-1G$_L$). GPho is converted to its active form via phosphorylation, by phosphorylase kinase, and is inactivated by dephosphorylation by protein phosphatase-1G (PP-1G).

The Regulation of Glycogen Metabolism

The processes involved in the regulation of glycogen metabolism in the liver are illustrated in figure 3.1 and a detailed review can be found in Bollen et al. (1998). A summary is provided here.

The synthesis and breakdown of glycogen is regulated by a pair of hormones, insulin and glucagon, which are produced in the β and α cells of the pancreas respectively. Insulin is released in response to elevated blood glucose and activates the storage of glucose as glycogen. Glucagon is secreted when blood glucose levels fall and promotes the breakdown of glycogen to release glucose. These hormones exert their influence on glycogen metabolism via a number of second messengers including cyclic adenosine monophosphate (cAMP), cAMP-dependent protein kinase (PKA), calcium (Ca^{2+}), and Akt (also known as protein kinase B (PKB)).

The main effect of insulin is to inactivate GSK3 via phosphorylation by Akt. This prevents the kinase from inactivating GSyn which allows glycogen synthesis to proceed. The mechanism of insulin action is described in greater detail in section 3.4 where the development of a mechanistic model of the pathway is described.

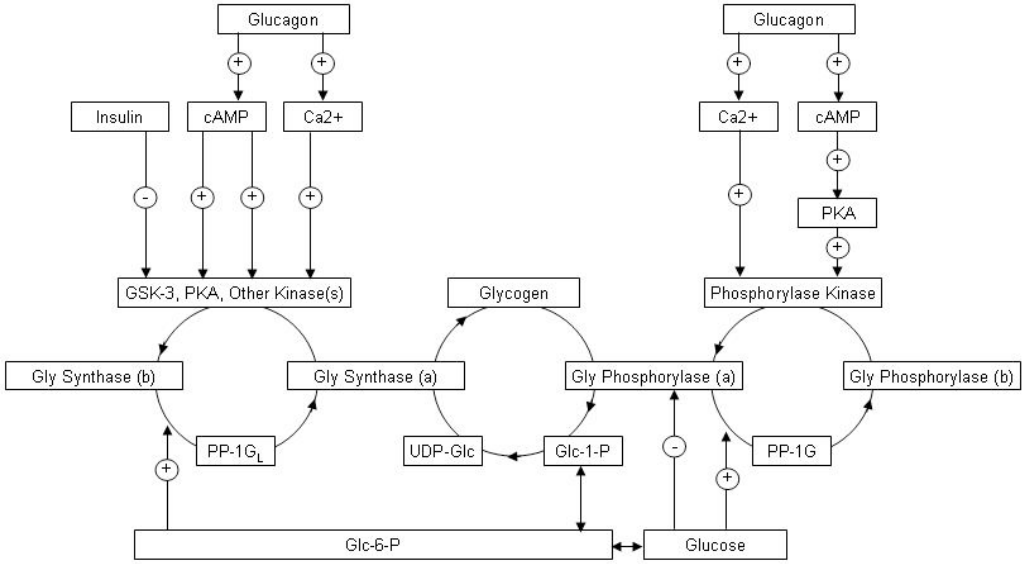


Figure 3.1: The regulation of glycogen metabolism

Glucagon binds to G-protein coupled receptors (GPCR) on the surface of liver cells. GPCRs are a class of receptors in which ligand binding causes a conformational change in the receptor that allows the associated G-proteins to be activated via GDP-GTP exchange. At least two classes of G-protein, G_s and G_q, are known to be coupled to glucagon receptors and are believed to be involved in two distinct signalling mechanisms (Jiang and Zhang, 2003).

The primary pathway involves G_s proteins and the activation of the enzyme adenylate cyclase. This leads to a large increase in the concentration of cAMP which binds to and activates PKA. This in turn phosphorylates a number of proteins including phosphorylase kinase. Phosphorylation of phosphorylase kinase increases its activity towards GPho, converting it to its active form. PKA also inhibits glycogen synthesis by increasing the inactivation of GSyn (Jiang and Zhang, 2003).

The second effect triggered by glucagon results in a rise in intracellular calcium however the mechanism by which this occurs is debated. Some studies have suggested that glucagon increases calcium via a cAMP-dependent mechanism (Staddon and Hansford, 1989). Other evidence points to a separate pathway triggered by activation of G_q proteins which regulate the activation of phospholipase C (PLC). PLC produces the second messenger inositol trisphosphate (IP3) which stimulates the release of calcium from intracellular stores (Hansen et al., 1998). Calcium effects glycogen regulation by causing a conformational change in phosphorylase kinase, enhancing its activity and causing more GPho to be converted to its active form. The end result is an increase in the rate of glycogen breakdown. Like PKA, calcium may also inhibit glycogen synthesis by

increasing the inactivation of GSyn. The contribution of this pathway to the regulation of glucose levels by glucagon is contentious (Aromataris et al., 2006).

Glycogen metabolism is also regulated directly by glucose (Cardenas and Goldbeter, 1996). Glucose binds to the active form of GPho inhibiting its activity and making it more susceptible to inactivation by dephosphorylation. This reduces the rate of glycogen breakdown. The presence of glucose, in the form of glucose-6-phosphate (Glc-6-P), also affects GSyn promoting its dephosphorylation to the active form. This results in an increased conversion of glucose into glycogen.

3.3 The Composite Model

The model discussed here was constructed using the modular approach developed during the UCL Beacon project. The model describes the regulation of blood glucose levels via the synthesis and breakdown of glycogen by the liver. It incorporates the effects of insulin, glucagon and glucose on glycogen metabolism and the feedback between the liver and the pancreas which generates the two regulatory hormones. The composite model consists of seven component models, which are described briefly below, connected via their inputs and outputs as shown in figure 3.2.

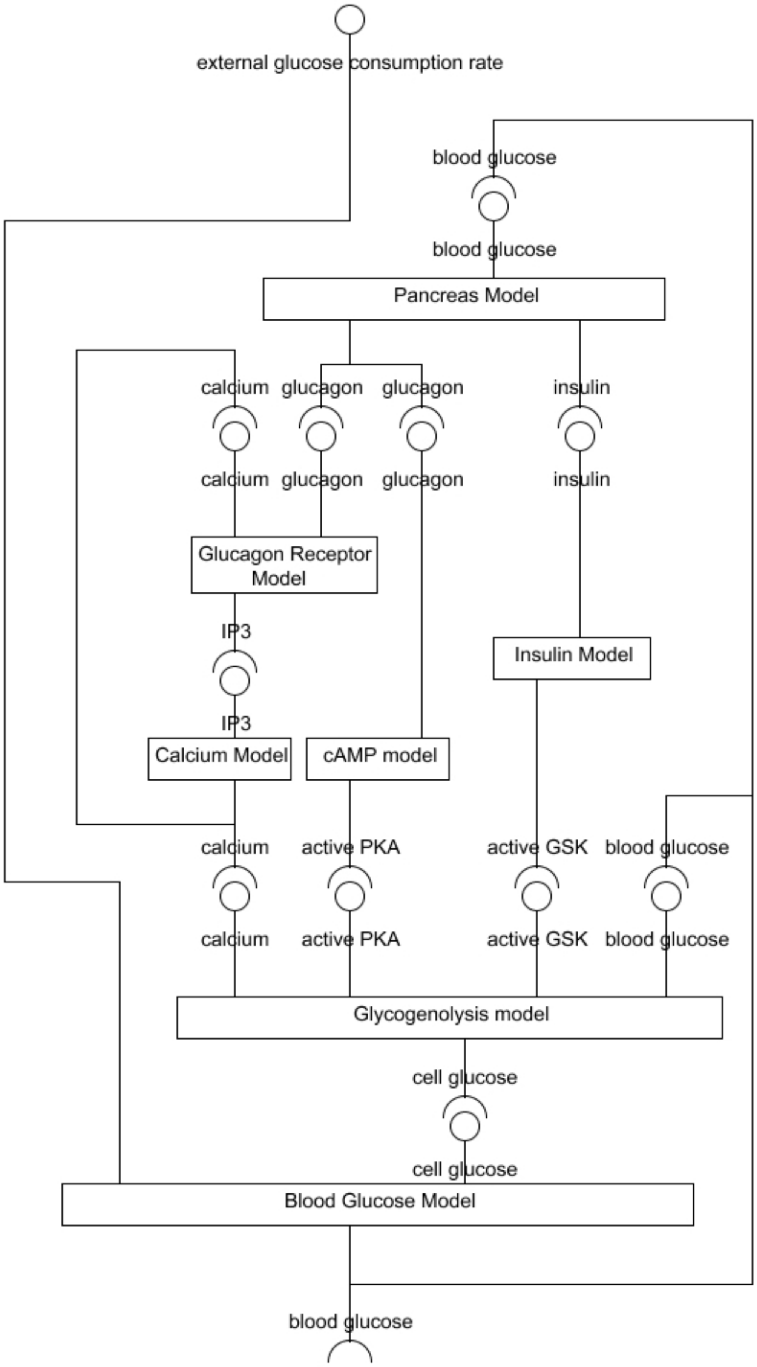


Figure 3.2: The structure of the model showing the seven components and their interactions. Model inputs are represented by circles, model outputs by arcs.

The models use a variety of different units depending on their origins. Where the connecting variables are in different units appropriate scaling must be carried out to ensure consistency. Where scalings are used these are discussed in section 3.3.8.

3.3.1 Pancreas Model

The pancreas model describes the production of the hormones glucagon, $L(t)$, and insulin, $I(t)$. The release of the hormones is determined by a time-delayed threshold response to the level of blood glucose, g_B, taken from the blood model. The outputs of the pancreas model, glucagon and insulin, provide inputs to the glucagon receptor, cAMP and insulin models. The model equations are presented below. The units of the pancreas model are arbitrary.

$$\frac{dL}{dt} = \frac{1}{\tau_L}\left\{\Theta_2\left(h(-x), t_{L_g}\right) - \frac{L}{L_{max}}\right\} \tag{3.1}$$

$$\frac{dI}{dt} = \frac{1}{\tau_I}\left\{\Theta_2\left(h(x), t_{I_g}\right) - \frac{I}{I_{max}}\right\} \tag{3.2}$$

$$x = \ln\left(\frac{g_B(t)}{g_{ref}}\right) \tag{3.3}$$

where $\Theta_n(x,t) = x^n/(x^n + t^n)$ (a Hill function) and $h(x) = x$ if $x \geq 0$ and 0 if $x < 0$. Therefore if $g_B(t)$ is below the reference value g_{ref} the pancreas releases glucagon and if $g_B(t) > g_{ref}$ insulin is produced. L_{max} and I_{max} define the maximum possible concentrations of glucagon and insulin.

Parameter Values

$g_{ref} = 2.5, L_{max} = 3, \tau_L = 1/2, t_{Lg} = 1/8, I_{max} = 4, \tau_I = 5/3, t_{Ig} = 1/2$

3.3.2 Glucagon Receptor Model

This model describes the activation of G_q proteins by glucagon which regulates the activation of PLC. Active PLC produces inositol trisphosphate (IP3) which acts as a second messenger in the mobilisation of intracellular calcium.

The model is based on previous mechanistic models described by Nauroschat and an der Heiden (1997) and Riccobene et al. (1999) and includes the following processes: ligand-receptor binding and dissociation; desensitisation of ligand bound receptors by phosphorylation and its dependence on active G-protein (Pitcher et al., 1992); the sequestration of receptors and its dependence on phos-

phorylation state; the activation of G-protein sub-units by ligand-bound receptors; the inactivation of G-proteins and its dependance on active PLC (Bourne and Stryer, 1992) and calcium dependent kinase (Sanchez-Bueno et al., 1990) which is modelled as a dependence on calcium (Kummer et al., 2000); the activation of PLC as a function of active G-protein. The model equations are:

$$\frac{dR_r}{dt} = k_{-1}LR_u - k_1L(t)R_r - k_sR_r + k_rR_s \tag{3.4}$$

$$\frac{dR_s}{dt} = k_{sp}LR_p + k_s(LR_u + R_r) - k_rR_s \tag{3.5}$$

$$\frac{dG_*}{dt} = K_{23}LR_uG - G_*\left(k_h + \frac{k_{cal1}}{k_{cal2}+G_*}C_0(t) + \frac{k_{plc1}}{k_{plc2}+G_*}PLC_*\right) \tag{3.6}$$

$$\frac{dLR_p}{dt} = k_p\left(1 + \frac{A_0G_*}{B_1+G_*}\right)\left(\frac{LR_u}{B_2+LR_u}\right) - k_{sp}LR_p \tag{3.7}$$

$$\frac{dPLC_*}{dt} = k_{PC}G_* - \frac{k_{PC1}PLC_*}{k_{PC2}+PLC_*} \tag{3.8}$$

where R_r, R_s, LR_u and LR_p are the free, sequestered, ligand bound and desensitised receptor concentrations respectively. G and G_* are the inactive and active G-protein concentrations, PLC_* is active PLC and C_0 is the calcium concentration. The following conservation constraints are also imposed:

$$G_0 = G + G_* \tag{3.9}$$

$$R_0 = R_r + R_s + LR_u + LR_p \tag{3.10}$$

where G_0 and R_0 are constants which represent the total G-protein and receptor concentrations. The model takes its inputs (glucagon (in μM) and calcium (in μM)) from the pancreas and calcium models. The model output is the concentration of active PLC (in μM). This is converted into an IP3 concentration which is used as an input to the calcium model.

Parameter Values

$k_{-1} = 10\text{s}^{-1}, k_1 = 100\mu\text{Ms}^{-1}, k_s = s_{sp} = 5.2\times10^{-3}\text{s}^{-1}, k_r = 4\times10^{-3}\text{s}^{-1}, K_{23} = 1\times10^{-7}\text{s}^{-1}, k_h = 0.2\text{s}^{-1}, k_{cal1} = 1.47\times10^3\mu\text{Ms}^{-1}, k_{cal2} = 3.54\times10^1, k_{plc1} = 2.19\times10^3\mu\text{Ms}^{-1}, k_{plc2} = 5.7, k_p = 6.5\times10^4\text{s}^{-1}, A_0 = 3, B_1 = 100, B_2 = 1\times10^6, k_{PC} = 6.06\times10^{-4}\text{s}^{-1}, k_{PC1} = 0.282, k_{PC2} = 0.255, R_0 = 126500, G_0 = 100000$

3.3.3 Calcium Model

Changes in the cytoplasmic calcium concentration (C_0) are a result of the following processes: the influx of calcium from the extracellular medium (J_{in}), the release of calcium from intracellular stores (J_{rel}), the removal of calcium from the cell by membrane pumps (J_{out}) and the reuptake of calcium into the internal stores (J_{SERCA}). In hepatocytes, the primary store of calcium is in the endoplasmic reticulum (ER). The release of calcium is triggered by binding of IP3 to receptors on the ER. The receptors are further activated by the increasing cytoplasmic calcium leading to calcium induced calcium release (CICR). High levels of calcium inhibit the receptors preventing further release. Excess calcium is pumped out of the cell by membrane bound pumps and resequestered back into the ER by the sarco/endoplasmic reticulum calcium ATPase (SERCA). These processes are described by a simplified version of the model of Hofer (1999):

$$\frac{dC_0}{dt} = \rho\left((J_{in} - J_{out}) + \alpha\left(J_{rel} - J_{SERCA}\right)\right) \tag{3.11}$$

$$\frac{dC_E}{dt} = \rho\alpha Vol\left(J_{SERCA} - J_{rel}\right) \tag{3.12}$$

$$J_{in} = v_0 + v_c\Theta_1\left(IP3, K_0\right) \tag{3.13}$$

$$J_{out} = v_4\Theta_2\left(C_0, K_4\right) \tag{3.14}$$

$$J_{SERCA} = v_3\Theta_2\left(C_0, K_3\right) \tag{3.15}$$

$$J_{rel} = \left(Uk_{1cal} + k_{2cal}\right)\left(C_E - C_0\right) \tag{3.16}$$

$$U\left(IP3, C_0\right) = \left[\Theta_1(IP3, d_p)\Theta_1(C_0, d_a)\Theta_1(Q, C_0)\right]^3 \tag{3.17}$$

$$Q(IP3) = d_2\frac{d_1 + IP3}{d_3 + IP3} \tag{3.18}$$

The model takes its input, the concentration of $IP3$, from the G-protein model. The output (calcium) is passed back to the G-protein model and also acts as an input into the glycogenolysis model. All concentrations are in units of μM.

Parameter Values

$\rho = 0.02\mu\text{m}^{-1}, \alpha = 2, Vol = 10, v_0 = 0.2\mu\text{Ms}^{-1}, v_c = 4.0\mu\text{Ms}^{-1}, v_3 = 9\mu\text{Ms}^{-1}, v_4 = 3.6\mu\text{Ms}^{-1}, K_0 = 4\mu\text{M}, K_3 = 0.12\mu\text{M}, K_4 = 0.12\mu\text{M}, k_{1cal} = 40\text{s}^{-1}, k_{2cal} = 0.02\text{s}^{-1}, d_a = 0.4\mu\text{M}, d_p = 0.2\mu\text{M}, d_1 = 0.3\mu\text{M}, d_2 = 0.4\mu\text{M}, d_a = 0.2\mu\text{M}$

3.3.4 cAMP Model

This model describes the primary signalling mechanism triggered by the binding of glucagon to G-protein coupled receptors, the cAMP dependent pathway. The activation of the receptors coupled to G_s proteins stimulates adenylate cyclase which in turn synthesises cAMP from ATP. cAMP subsequently activates the cAMP-dependent protein kinase also known as PKA. The production of cAMP and the activation of PKA are modelled as threshold functions of the hormone concentration and cAMP respectively. The model also includes the potential nuclear localisation of PKA.

$$\frac{dA}{dt} = k_{Abkg} + k_A\Theta_{n_R}(L(t), t_R) - k_{Adeg}A \tag{3.19}$$

$$\frac{dP}{dt} = k_{-a}P_* - k_a\Theta_{n_A}(A, t_A)P \tag{3.20}$$

$$\frac{dP_{N*}}{dt} = k_N P_*\Theta_{n_N}(A, t_{N*}) - k_{NA}P_{N*} \tag{3.21}$$

where A is cAMP, P is the fraction of inactive PKA, P_* is the fraction of active PKA, P_{N*} is the fraction of active PKA in the nucleus and $P + P_* + P_{N*} = 1$.

Parameter Values

$k_{Abkg} = 1.2\text{hours}^{-1}, k_A = 5\text{hours}^{-1}, n_2, t_R = 1, k_{Adeg} = 4\text{hours}^{-1}, k_{-a} = 1\text{hours}^{-1}, k_a = 99\text{hours}^{-1}, n_A = 8, t_A = 1, k_N = 0.16\text{hours}^{-1}, k_{NA} = 0.16\text{hours}^{-1}, n_N = 8, t_N = 1$

3.3.5 Insulin Model

The insulin model describes the inactivation of GSK3 by insulin. The pathway from the hormone to the kinase involves numerous steps (see section 3.4) however the model used here simply represents the inactivation of GSK3 as a time-delayed threshold response to the concentration of insulin via a single equation. The units of both insulin and GSK3 are arbitrary with unit insulin taken to be the maximum insulin concentration possible. The parameters of the model were selected to reproduce experimental observations.

$$\frac{dGSK3}{dt} = \frac{1}{\tau_{GSK3}}\left[\Theta_n\left(I\left(t\right), t_I\right) - GSK3\right] \tag{3.22}$$

Parameter Values

$\tau_{GSK3} = 1.0, t_I = 0.5, n = 8$

3.3.6 Blood Transport Model

The blood transport model describes the transport of glucose between the blood and the liver. Glucose is transported into liver cells by the passive membrane glucose transporter GLUT2 where it can be converted into Glc-6-P which can not be transported out of the cell. However, in this and the glycogenolysis model (section 3.3.7) the total cellular glucose and Glc-6-P concentration is represented by a single variable g_C. The use of a single passive transport term would overestimate the efflux of calcium from the cell. This is avoided by the inclusion of an additional influx term. The modified model fits the data from a perfusive radiolabelling experiment in pigs (Munk et al., 2001).

The blood model also includes a glucose drive term, $M(t)$ which represents an external glucose source or sink. A positive value indicates a glucose input, for example feeding, and a negative value represents increased glucose utilisation, for example exercise. The blood glucose concentration is in units of μM.

$$\frac{dg_B}{dt} = M(t) - k_{pg}g_B + k_{cg}(g_C(t) - g_B) \tag{3.23}$$

Parameter Values

$k_{pg} = 0.003\text{s}^{-1}, k_{cg} = 0.006\text{s}^{-1}$

3.3.7 Glycogenolysis Model

This model describes the synthesis and breakdown of glycogen by the liver and the consequent change in the cellular glucose concentration (in μM). The rate of change of glycogen, G, and cellular glucose, g_C are given by:

$$\frac{dG}{dt} = Syn - Brk \tag{3.24}$$

$$\frac{dg_C}{dt} = k_{pg}g_B - k_{cg}(g_C - g_B) - Syn + Brk \tag{3.25}$$

The terms Syn and Brk describe the synthesis and breakdown of glycogen and are given by:

$$Syn = k_{Syn} Sta \left[\left(\frac{1}{g_C} \right)^n + \left(\frac{1}{Glu_s} \right)^n \right]^{-1/n} \tag{3.26}$$

$$Brk = k_{Brk} Pho \left[\left(\frac{1}{G} \right)^n + \left(\frac{1}{Gly_s} \right)^n \right]^{-1/n} \tag{3.27}$$

k_{Syn} and k_{Brk} are the maximum rates of synthesis and breakdown, Sta and Pho are the relative activities of glycogen synthase and glycogen phosphorylase and the final terms describe the increase in synthesis (breakdown) with the level of glucose (glycogen) up to some saturating value Glu_s (Gly_s). The activities of glycogen synthase and phosphorylase are given by:

$$\frac{dSta}{dt} = \frac{1}{\tau_{Sta}} (Sta_{inf} - Sta) \tag{3.28}$$

$$\frac{dPho}{dt} = \frac{1}{\tau_{Pho}} (Pho_{inf} - Pho) \tag{3.29}$$

The activities of glycogen synthase and phosphorylase are regulated by a number of factors (see section 3.2.1 and figure 3.1). The following factors are included here: active PKA, calcium, inactive GSK3 and glucose. Rather than modelling the numerous biological processes involved in the regulation the model uses the following logical operators:

$$AND : x \wedge y = xy, \;\; OR : x \vee y = x + y - xy, \;\; NOT :\sim x = 1 - x \tag{3.30}$$

together with hill functions of each of the variables to produce fuzzy logic statements that determine the activity of glycogen synthase and phosphorylase:

$$Pho_{inf} = [\Theta_n(PKA, t_{PKA}) \vee \Theta_n(C, t_C) \vee \sim \Theta(g_C, t_{Glu})] \wedge \sim \Theta_n(GSK3, t_{GSK3}) \tag{3.31}$$

$$Sta_{inf} = \sim [\Theta_n(PKA, t_{PKA}) \vee \Theta_n(C, t_C)] \wedge \Theta_n(g_C, t_{Glu}) \vee \Theta_n(GSK3, t_{GSK3}) \tag{3.32}$$

These statements represent the following qualitative features:

1. Pho_{inf} increases as active PKA or calcium increase and decreases as glucose or inactive GSK3 increase

2. Sta_{inf} decreases as active PKA or calcium increase and increases as glucose or inactive GSK3 increase

Parameter Values

$k_{Syn} = 0.025\text{s}^{-1}, Glu_s = 1000\mu\text{M}, k_{Brk} = 0.1\text{s}^{-1}, Gly_s = 1000\mu\text{M}, \tau_{Pho} = 60\text{s}, t_{PKA} = 0.5, t_C = 0.3\mu\text{M}, t_{Glu} = 4781\mu\text{M}, t_{GSK3} = 0.5$

3.3.8 Scalings

Where the models are constructed in different units it is necessary to use appropriate scaling values to ensure consistency between connecting variables (see figure 3.2). The selected values are shown in table 3.1 and discussed below. Where no value is given no scaling is required.

From		**To**		
Variable	**Model**	**Variable**	**Model**	**Value**
Glucose	Blood	Glucose	Pancreas	2.5/4500
PLC	Glucagon Receptor	IP3	Calcium	100
Glucagon	Pancreas	Glucagon	cAMP	0.8
Glucagon	Pancreas	Glucagon	Glucagon Receptor	0.033
Insulin	Pancreas	Insulin	Insulin	0.25

Table 3.1: Values of scaling parameters used to ensure consistency between the connecting variables in the composite model.

The scaling of the glucose concentration between the blood and pancreas models is chosen so that the reference level in the pancreas, $g_{ref} = 2.5$, at which the pancreas produces neither glucagon or insulin is equivalent to a plausible level in the blood. This was chosen to be 80mg/dl or 4500μM.

The active PLC concentration in the glucagon receptor model and the IP3 variable in the calcium model are assumed to be proportional, with 100μM IP3 corresponding to 1μM active PLC. This value was chosen so that the IP3 variable takes values in the range expected in calcium oscillation modelling, given the range of values of PLC produced by the receptor model.

The scaling factors between the output of the pancreas (arbitrary units of glucagon and insulin) are chosen to ensure that the variables take appropriate values in the cAMP, glucagon receptor and insulin models. The units of glucagon in the cAMP model are defined in units of the hormone receptor threshold, $t_R = 1$. A scaling value for glucagon between the pancreas and cAMP models of 0.8 was chosen to give appropriate values relative to the threshold. In the glucagon receptor model, the concentration of glucagon is defined in μM. The scaling factor is selected to ensure that the variable takes values in the physiological range in the glucagon receptor model. In the insulin model, the concentration of insulin is taken to vary between 0 and 1 units (assuming

normal pancreas function). The scaling value is chosen so that the maximum insulin concentration produced by the pancreas model ($I_{max} = 4$) corresponds to unity in the insulin model.

3.3.9 Discussion

The composite model has previously been shown to display the expected qualitative behaviour in response to a range of glucose challenges. It is also able to reproduce the results of a glucagon challenge, the administration of a bolus of glucagon in a healthy patient (Lockton and Poucher, 2007). In addition an investigation of the effects of varying the insulin sensitivity (t_I in the insulin component model) on the model has identified a number of hypotheses about the operation of the homeostatic system (Hetherington et al., 2009). The model also serves as a proof of concept for the modular construction approach which allows published models to be re-used and components to be easily replaced with alternative representations.

A number of the original component models are simplified descriptions based on empirical observations rather than a mechanistic knowledge of the biology. For example, while the processes triggered by glucagon are modelled in detail the effects of insulin are described by a single equation. While the simple insulin model is able to reproduce the inactivation of GSK3 observed experimentally the lack of mechanistic detail limits its utility in analysing and understanding the system. As discussed in the introduction to this chapter, the insulin signalling pathway is a key component of the glucose regulatory system and defects in the pathway are important in the development of type 2 diabetes. Modelling this pathway in more detail will allow us to investigate the effects of perturbations of the pathway on the system behaviour. The remainder of this chapter describes the development of a more detailed model of the insulin pathway.

3.4 The Insulin Signalling Pathway

Insulin has a number of effects including regulation of metabolism, cell growth and cell differentiation. The actions of insulin are initiated when the hormone binds to its cell surface receptors triggering a signalling pathway which has pleiotropic effects in virtually all tissues (Plum et al., 2006). Figure 3.3 shows the known components of the insulin signalling pathway and their interactions. There are three processes which relate directly to glucose homeostasis: glucose uptake, control of glycolysis and gluconeogenesis and the regulation of glycogen synthesis.

In muscle and adipose tissue insulin increases glucose uptake by stimulating the translocation of the hexose transporter, GLUT4, to the plasma membrane (Saltiel and Kahn, 2001). In liver cells, the primary glucose transporter GLUT2 is not insulin-dependent so insulin does not affect the rate

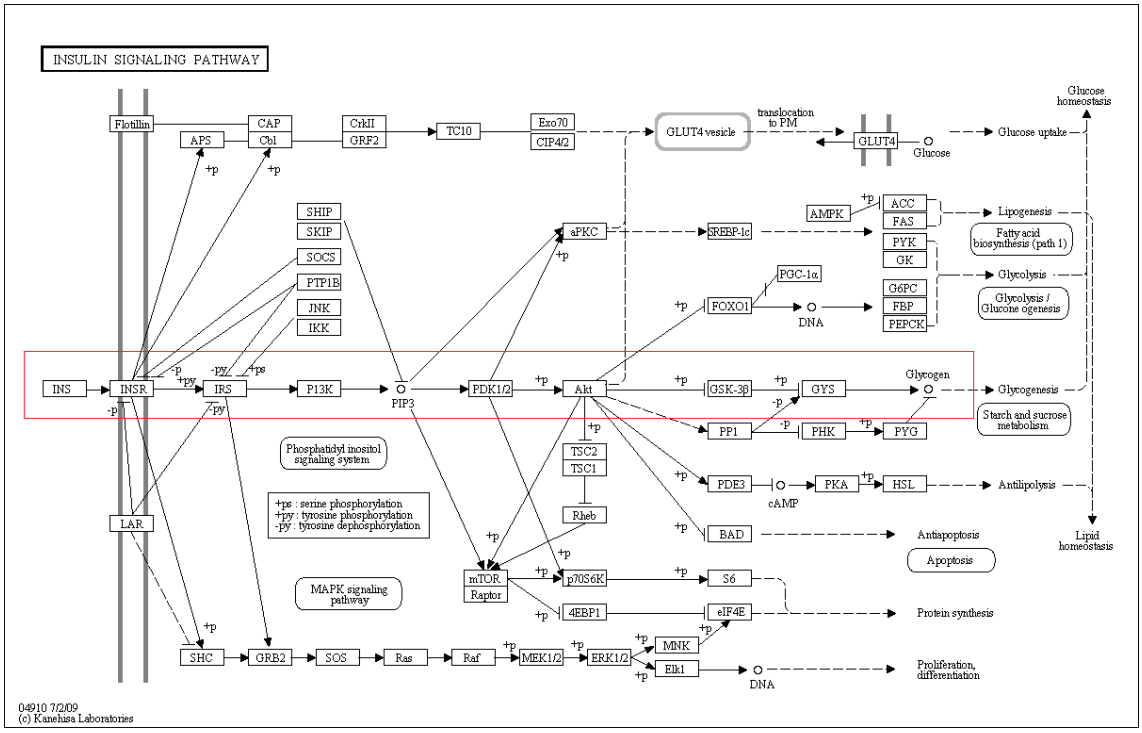


Figure 3.3: Insulin signalling pathway taken from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto, 2000). The pathway from insulin to the inactivation of GSK3 is shown in the red box.

of glucose uptake in hepatocytes. The second effect of insulin is the suppression of endogenous hepatic glucose production (gluconeogenesis). This occurs at the level of gene transcription by downregulating the production of enzymes necessary for the production of glucose from pyruvate (Desvergne et al., 2006). It involves two main transcription factors, sterol response element binding protein 1c (SREBP-1c) and FOXO1 (a member of the forkhead transcription factor family) (Carter and Brunet, 2007). The third effect, and the one on which I will focus, is the effect of insulin on the rate of glycogen synthesis. The pathway from insulin to glycogen is highlighted by the red box in 3.3 and is described below.

The insulin receptor (INSR) is one of a family of receptor tyrosine kinases (RTK). These are tetrameric proteins consisting of two extracellular α-subunits and two transmembrane β-subunits (Saltiel and Kahn, 2001). In the absence of insulin (INS), the α-subunit inhibits the tyrosine kinase in the intracellular portion of the β-subunit. Upon binding of the hormone, a conformational change occurs which relieves the inhibition of the tyrosine kinase activity. Subsequently the kinase in one β-subunit phosphorylates the other half of the receptor dimer. This autophosphorylation results in a large increase in the catalytic activity of the receptor (Nystrom and Quon, 1999). The tyrosine kinase activity of the insulin receptor is negatively regulated by dephosphorylation of the receptor

by protein tyrosine phosphatases (PTPs) (Drake and Posner, 1998).

There are at least nine substrates of the insulin receptor four of which are varieties of the insulin-receptor substrate (IRS) protein (White, 1998). IRS proteins are phosphorylated by the kinase activity of the receptor and act as docking sites for molecules which contain specific sequences known as SH2 domains. One such molecule is phosphoinositide 3-kinase (PI3K) which consists of a regulatory p85 subunit and a catalytic p110 subunit. The p85 unit contains an SH2 domain and is recruited to the plasma membrane of the cells by the activated IRS. This places it in the vicinity of its physiological substrate, phosphatidylinositol (4,5) bisphosphate (PI(4,5)P2) which it phosphorylates to produce PI(3,4,5)P3 (PIP3) (Lizcano and Alessi, 2002).

PIP3 binds to the pleckstrin homology (PH) domains of a variety of signalling molecules modifying their activity and intracellular location. Two such molecules are phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (PKB) also known as Akt. The co-localisation of these molecules allows PDK1 to phosphorylate and activate Akt. There is also evidence that a second kinase (referred to as PDK2) is also involved in the phosphorylation of Akt (Chan and Tsichlis, 2001; Dong and Liu, 2005). Akt in turn phosphorylates and inactivates glycogen synthase kinase (GSK3). It is the inactivation of this kinase that allows glycogen synthesis to proceed at an increased rate (see section 3.2.1).

3.5 Modelling the Insulin Signalling Pathway

In their 2002 paper, Sedaghat et al. (2002) presented a mathematical model of the insulin signalling pathway. Their goal was to investigate the mechanisms by which insulin causes increased glucose uptake in muscle. Their model produced good agreement with experimental results and has been used in a number of further studies (Giri et al., 2004; Hori et al., 2006; Liu et al., 2009). It is the most complete model of the insulin signalling pathway to be published to date.

It can be seen in figure 3.3 that many of the steps from insulin to GLUT4 are shared with the pathway from insulin to GSK3. Consequently, it was decided to use the Sedaghat model (Sedaghat et al., 2002) as the basis for a model of the inactivation of GSK3 by insulin.

3.5.1 Sedaghat Model

The Sedaghat model is constructed from three previously published and validated models (a description of the insulin receptor binding kinetics (Wanant and Quon, 2000), a model of receptor recycling (Quon and Campfield, 1991) and a model of GLUT4 translocation (Quon, 1994)) coupled with a description of the intracellular signalling pathway. Here we will reuse the first two

components together with a modified representation of the post receptor signalling pathway which describes the additional steps in the inactivation of GSK3 which were not included in the published model.

Receptor Binding Subsystem

The receptor binding subsystem represents the association and dissociation of insulin and the phosphorylation and dephosphorylation of the receptor. Free receptors (x_2) can bind a single insulin molecule (x_1). The ligand-receptor complex (x_3) then undergoes phosphorylation. The phosphorylated, once-bound receptor (x_5) can bind a second insulin molecule (which has no effect on the phosphorylation state) resulting in a twice-bound phosphorylated receptor (x_4). The dissociation of the first insulin molecule leads to rapid dephosphorylation of the receptor. These processes are represented schematically at the top of figure 3.4.

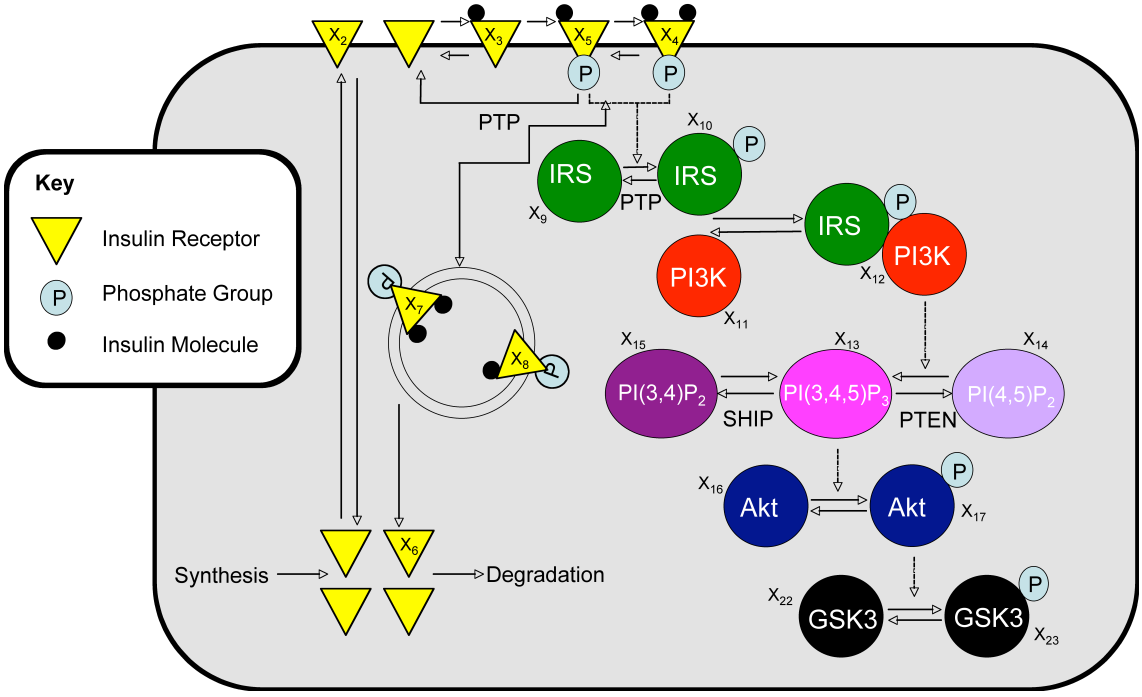


Figure 3.4: Schematic of the processes included in the insulin model. Triangles represent the various states of the insulin receptor, $x_2 - x_8$. The circles represent the species in the post receptor signalling pathway which are included as state variables in the model. Phosphorylated (P) and unphosphorylated forms are modelled as separate species. IRS = insulin receptor substrate, PI3K = phosphoinositide 3-kinase, $PI(3,4)P_2$, $PI(3,4,5)P_3$ and $PI(4,5)P_2$ are phophoinositol lipids, Akt = protein kinase B, GSK3 = glycogen synthase kinase.

Receptor Recycling Subsystem

The second subsystem describes the synthesis, degradation, exocytosis (transfer to cell membrane) and endocytosis (internalisation) of receptors. Free receptors are recycled directly into the internal pool (x_6) which undergoes constant turnover via receptor synthesis and degradation. Internalised phosphorylated receptors (x_7 (twice bound) and x_8 (once bound)) undergo an additional step in which they are dephosphorylated before they are added to the intracellular pool. The receptor recycling subsystem is shown down the left hand side of figure 3.4. The equations for the receptor binding and recycling subsystems are shown below:

$$\frac{dx_2}{dt} = k_{-1}x_3 + k_{-3}x_5 - k_1x_1x_2 + k_{-4}x_6 - k_4x_2 \tag{3.33}$$

$$\frac{dx_3}{dt} = k_1x_1x_2 - k_{-1}x_3 - k_3x_3 \tag{3.34}$$

$$\frac{dx_4}{dt} = k_2x_1x_5 - k_{-2}x_4 + k_{-4'}x_7 - k_{4'}x_4 \tag{3.35}$$

$$\frac{dx_5}{dt} = k_3x_3 + k_{-2}x_4 - k_2x_1x_5 - k_{-3}x_5 + k_{-4'}x_8 - k_{4'}x_5 \tag{3.36}$$

$$\frac{dx_6}{dt} = k_5 - k_{-5}x_6 + k_6(x_7 + x_8) + k_4x_2 - k_{-4}x_6 \tag{3.37}$$

$$\frac{dx_7}{dt} = k_{4'}x_4 - k_{-4'}x_7 - k_6x_7 \tag{3.38}$$

$$\frac{dx_8}{dt} = k_{4'}x_5k_{-4'}x_8 - k_6x_8 \tag{3.39}$$

The receptor synthesis rate k_5 is defined so that the net synthesis and degradation of receptors is zero under basal conditions therefore $k_5 = k_{-5}x_6(0)$. If the intracellular receptor concentration falls below its basal level an accelerated synthesis rate $k_{5acc} = 6k_5$ is used.

Post Receptor Signalling Pathway

The Sedaghat model includes a description of the pathway from the insulin receptor to the activation of Akt. It is assumed to be a closed system and the synthesis and degradation of signalling molecules is not represented. The processes included in the model are shown in figure 3.4.

IRS (x_9) is activated (x_{10}) by the phosphorylated receptors and deactivated by PTP. The rate of IRS activation is modelled as a linear function of the phosphorylated receptor concentration ($x_4 + x_5$). Activated IRS binds with and activates free PI3K (x_{11}) in a 1:1 stoichiometry. This complex (x_{12}) converts PI(4,5)P2 (x_{14}) to PI(3,4,5)P3 (x_{13}). This phophoinositol lipid is also generated from PI(3,4)P2 (x_{15}). The lipid phosphatases, SHIP2 and PTEN convert PI(3,4,5)P3

back to PI(3,4)P2 and PI(4,5)P2 respectively. The activation of Akt ($x_{16} \rightarrow x_{17}$) is taken to be dependent on the level of PI(3,4,5)P3 and any intermediate steps (e.g. the action of PDK1/2) are not modelled.

$$\frac{dx_9}{dt} = k_{-7}x_{10} - \frac{k_7 x_9(x_4 + x_5)}{IR_p} \tag{3.40}$$

$$\frac{dx_{10}}{dt} = \frac{k_7 x_9(x_4 + x_5)}{IR_p} + k_{-8}x_{12} - (k_{-7} + k_8 x_{11})x_{10} \tag{3.41}$$

$$\frac{dx_{11}}{dt} = k_{-8}x_{12} - k_8 x_{10}x_{11} \tag{3.42}$$

$$\frac{dx_{12}}{dt} = k_8 x_{10}x_{11} - k_{-8}x_{12} \tag{3.43}$$

$$\frac{dx_{13}}{dt} = k_9 x_{14} + k_{10}x_{15} - (k_{-9} + k_{-10})x_{13} \tag{3.44}$$

$$\frac{dx_{14}}{dt} = k_{-9}x_{13} - k_9 x_{14} \tag{3.45}$$

$$\frac{dx_{15}}{dt} = k_{-10}x_{13} - k_{10}x_{15} \tag{3.46}$$

$$\frac{dx_{16}}{dt} = k_{-11}x_{17} - k_{11}x_{16} \tag{3.47}$$

$$\frac{dx_{17}}{dt} = k_{11}x_{16} - k_{-11}x_{17} \tag{3.48}$$

The rate at which PI(4,5)P2 is converted to PI(3,4,5)P3, k_9, is taken to be a linear function of active PI3K, (x_{12}), increasing from some basal value in the absence of insulin to k_{9stim} at maximal stimulation. k_{-9} and k_{9basal} are also defined in terms of k_{9stim}.

$$k_9 = ((k_{9stim} - k_{9basal})\frac{x_{12}}{PI3K_{max}} + k_{9basal}) \tag{3.49}$$

The rate of activation of Akt, k_{11}, is taken to be a function of PI(3,4,5)P3, (x_{13}), increasing from zero to its maximal value as PI(3,4,5)P3 increases from its basal value, $x_{13}(0)$ to its maximal value $PIP3_{max}$.

$$k_{11} = k_{11d}\frac{(x_{13} - x_{13}(0))}{(PIP3_{max} - x_{13}(0))} \tag{3.50}$$

Initial Conditions and Parameter Values

The initial conditions and parameter values for the model taken from (Sedaghat et al., 2002) are listed in tables 3.2 and 3.3.

Variable	Description	Value	Units
x_2	Unbound surface IR	9×10^{-13}	M
x_3	Unphosphorylated once-bound surface IR	0	M
x_4	Phosphorylated twice-bound surface IR	0	M
x_5	Phosphorylated once-bound surface IR	0	M
x_6	Unphosphorylated unbound intracellular IR	1×10^{-13}	M
x_7	Phosphorylated twice-bound intracellular IR	0	M
x_8	Phosphorylated once-bound intracellular IR	0	M
x_9	Unphosphorylated IRS	1×10^{-12}	M
x_{10}	Tyrosine-phosphorylated IRS	0	M
x_{11}	Inactivated PI3K	1×10^{-13}	M
x_{12}	Active IRS/PI3K complex	0	M
x_{13}	PI(3,4,5)P3 in total lipid population	0.31	% of total lipid
x_{14}	PI(4,5)P2 in total lipid population	99.4	% of total lipid
x_{15}	PI(3,4)P2 in total lipid population	0.29	% of total lipid
x_{16}	Inactivated Akt	100	% of total Akt
x_{17}	Activated Akt	0	% of total Akt

Table 3.2: Initial conditions used in the insulin model. Abbreviations: IR=insulin receptor.

Parameter	Reaction	Value	units
k_1	Association rate of first insulin molecule to IR	6×10^7	$M^{-1}\ min^{-1}$
k_{-1}	Dissociation rate of first insulin molecule from IR	0.20	min^{-1}
k_2	Association rate of second insulin molecule to IR	6×10^7	$M^{-1}\ min^{-1}$
k_{-2}	Dissociation rate of second insulin molecule from IR	20	min^{-1}
k_3	Phosphorylation rate of surface IR	2500	min^{-1}
k_{-3}	Dephosphorylation rate of surface IR	0.20	min^{-1}
k_4	Endocytosis of free IR	0.00033	min^{-1}
k_{-4}	Exocytosis of free IR	0.003	min^{-1}
$k_4^{'}$	Endocytosis of bound IR	2.1×10^{-3}	min^{-1}
$k_{-4}^{'}$	Exocytosis of bound IR	2.1×10^{-4}	min^{-1}
k_{-5}	IR degradation	1.67×10^{-18}	min^{-1}
k_6	Dephosphorylation of intracellular IR	0.461	min^{-1}
k_7	Phosphorylation of IRS	4.16	min^{-1}
k_{-7}	Dephosphorylation of IRS	1.396	min^{-1}
k_8	Formation of IRS/PI3K complex	0.706×10^{12}	min^{-1}
k_{-8}	Separation of IRS/PI3K complex	10	min^{-1}
k_{9stim}	Maximal conversion of PI(4,5)P2 to PI(3,4,5)P3	1.39	min^{-1}
k_{11d}	Maximal phosphorylation of Akt	$\ln(2)$	min^{-1}
k_{-11}	Dephosphorylation of Akt	$10\ln(2)$	min^{-1}

Table 3.3: Nominal parameter values for the insulin model.

3.5.2 Modelling the Inactivation of GSK3

Glycogen synthase kinase (GSK3) regulates glycogen synthesis by inactivating glycogen synthase (GSyn). Insulin inactivates GSK3, reducing the inactivation of GSyn resulting in an increased rate of glycogen synthesis and a lowering of the blood glucose level. GSK3 is inactivated via phosphorylation by Akt, the activity of which is described by the published insulin signalling model described above.

To describe the inactivation of GSK3 two new variables representing the active and inactive percentage of the total GSK3 concentration were added to the model. The rates of change of these variables are described by equations 3.51 and 3.52:

$$\frac{dx_{22}}{dt} = k_{-15}x_{23} - k_{15}x_{22} \tag{3.51}$$

$$\frac{dx_{23}}{dt} = k_{15}x_{22} - k_{-15}x_{23} \tag{3.52}$$

where x_{22} is the percentage of active GSK3, x_{23} is the percentage of inactive GSK3, k_{15} is the rate of phosphorylation (inactivation) of GSK3 by Akt and k_{-15} is the rate at which GSK3 is dephosphorylated.

Initial Conditions

It is assumed that under basal conditions (no insulin) all GSK3 is active so that $x_{22}(0) = 100\%, x_{23}(0) = 0\%$. This follows from the assumption in (Sedaghat et al., 2002) that at basal conditions no Akt is in the phosphorylated state.

Rate Constants

The half-time ($t_{1/2}$) for inhibition of GSK3 by insulin is approximately 2 minutes (Hurel et al., 1996; Cross et al., 1997). For a first order rate constant (Sedaghat et al., 2002):

$$k = \frac{[\ln(2)]}{t_{1/2}} \tag{3.53}$$

Using equation 3.53 we can define $k_{15} = k_{15d} = [\ln(2)]/2$ min^{-1} at equilibrium following maximal insulin stimulation. It is also assumed that maximal insulin stimulation produces a 60:40 ratio of inactive to active GSK3 (Cross et al., 1997) so that at equilibrium when $k_{-15}x_{23} = k_{15}x_{22}$:

$$k_{-15} = k_{15}/1.5 \tag{3.54}$$

This means that we can constrain $k_{-15} = [\ln(2)]/3$. The rate at which GSK3 is inactivated depends on the activity of Akt. It is assumed that this rate, k_{15} increases from 0 to $k_{15d} = \ln(2)/2$ as a linear function of the amount of activated Akt:

$$k_{15} = k_{15d}\frac{x_{17}}{Akt_P^{max}} \tag{3.55}$$

where Akt_P^{max} is the percentage of phosphorylated Akt following maximal insulin stimulation.

3.5.3 Model Validation

The published part of the insulin model described above was previously validated against experimental data taken from the literature. The dynamics of IRS phosphorylation, PI3K activation, PI(3,4,5)P3 production and Akt phosphorylation were found to show good agreement with experimentally measured timecourses. The extended model has been validated by comparison with both published data and experiments conducted in collaboration with members of the Beacon project.

Initial assessments of the modified model were performed against data taken from van Weeren et al. (1998). The experimental conditions were replicated by applying a constant insulin input of magnitude $1\mu g.ml^{-1}$ at $t = 0$ until $t = 7$ mins at which point the stimulus was removed. Figure 3.5 shows the comparison between the model and data. It can be seen that the model shows reasonable qualitative agreement with the data, replicating both the inactivation of GSK3 in response to insulin and the reactivation upon removal of the hormone.

To further validate the model, experiments were conducted to measure the inactivation timecourse of GSK3 in rat hepatocytes in response to varying insulin doses. The left hand panel of figure 3.6 shows the timecourse predicted by the model and the right hand panel shows the experimental data.

The model displays the same dose dependent rise in phosphorylated GSK3 observed in the experimental time-course. For 10nM and 100nM both the model and experimental data show that

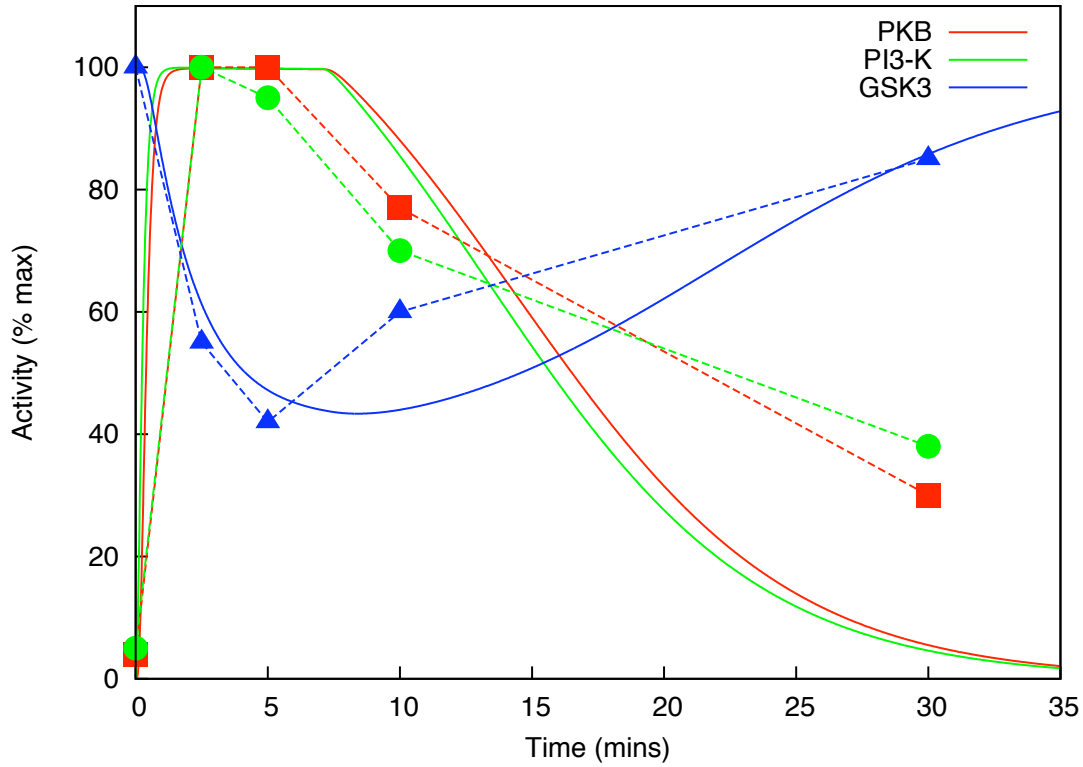
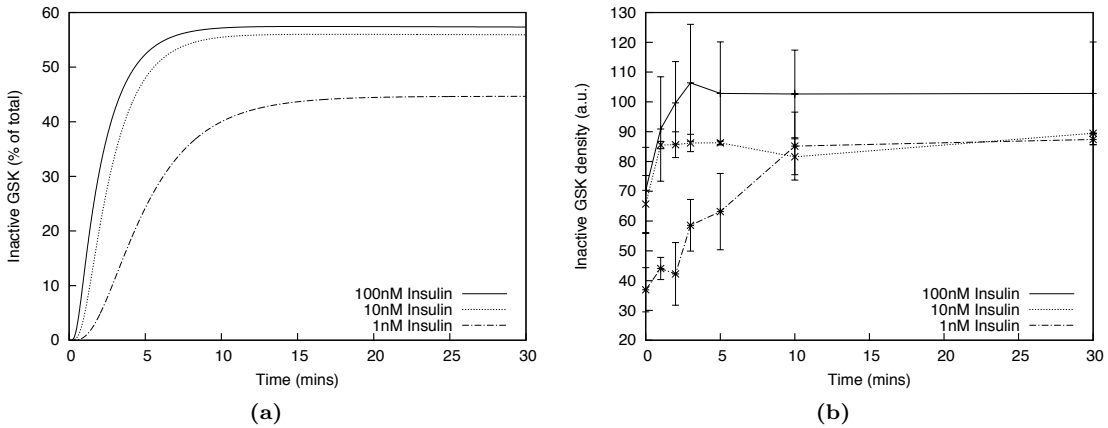


Figure 3.5: Initial comparison of the model output and experimental data. Experimental data taken from (van Weeren et al., 1998) is shown by data points joined by dashed lines. The model prediction is shown by solid lines. In each case the system is stimulated by the addition of insulin ($1\mu g.ml^{-1}$) for 7 minutes at $t = 0$



(a) (b)

Figure 3.6: Timecourse of GSK3 phosphorylation in response to insulin. Panel a shows the output of the mathematical model in response to a constant input of 1, 10 and 100nM insulin. Panel b shows the phosphorylation of GSK3 measured in isolated rat hepatocytes following addition of 1, 10 and 100nM insulin at t=0.

maximal phosphorylation is reached after approximately 5 minutes. At 1nM insulin the rate of phosphorylation is decreased (maximal phosphorylation is achieved at approximately 10 minutes) and a lower maximum state is reached.

3.6 Discussion

This chapter has described a composite multi-scale model of blood glucose regulation which focuses on the role of the liver in storing excess glucose as glycogen. The model is constructed by joining together seven component models which describe various aspects of the complete system. The composite model has previously been shown to reproduce the expected qualitative system level behaviour in response to both glucose and glucagon challenges. The effects of varying insulin sensitivity (via the threshold for insulin to inactivate GSK3, t_I in the simple insulin model) have also previously been studied.

The sub-models of the original model were constructed at different levels of detail depending on the availability of existing models and biological knowledge. The lack of detail in certain models limits their usefulness in understanding the function of the system. This lack of detail was particularly apparent in the insulin model. To address this issue a mechanistic model of the insulin signalling pathway was identified from the literature and modified to describe the inactivation of GSK3 by insulin. The model has been shown to provide good agreement with experimental data on the inactivation of GSK3 in liver cells.

The parameters of the component models have been taken from the literature, derived from experimental observations or tuned by fitting or comparison of the model to data. As a result the majority of the parameters have some associated uncertainty. It is important to investigate how this uncertainty affects the model output. More generally, studying how variation in the parameters effects the model behaviour can aid our understanding of the biological system and suggest how its behaviour can be modified or controlled. Global sensitivity analysis techniques provide a way to address these questions in a systematic manner.

The rest of this thesis describes the development of sensitivity analysis techniques suitable for the study of composite multi-scale biological models using the components of the glucose homeostasis model as examples. The methods will be applied to the complete model to investigate the importance of the various sub-processes and reactions in the regulation of blood glucose.

Chapter 4

Global Sensitivity Analysis of Time Dependent Model Outputs

This chapter describes a new approach to the application of sensitivity analysis techniques to time dependent model outputs. It introduces the use of principal component analysis to automatically identify the major modes of variation in a set of model outputs and shows how this technique can be combined with two SA methods, the global variance decomposition method of Sobol and Morris' screening design, to investigate the sensitivity of those modes to the model parameters. An "overall" sensitivity, which measures the effect of a parameter on the complete model output, is also defined. The approach is demonstrated on two of the component models of the glucose homeostasis system.

4.1 Introduction

As discussed in chapter 2 much of the sensitivity analysis (SA) literature is focussed on analysis of scalar model outputs (Saltelli et al., 2000a). This is true of many applications of SA in biology. For example, in metabolic control analysis (MCA) the focus is on the sensitivity of the steady state concentrations or fluxes to variations in the system parameters. However in many biological systems it is the transient or dynamic behaviour which is of particular interest and we therefore require methods which are appropriate for dealing with time dependent model output.

The typical approach to the analysis of such systems is to calculate sensitivities at multiple time-points along the output trajectory. However, often we are interested in how the form or shape of the model output depends on the parameters. This information is not well captured by time-varying sensitivities. To address this problem a set of scalar values which describe the key features of the model output can be defined and used as the output for any SA method. The drawback of this approach is that it is problem specific. We must select an appropriate set of

features, which typically requires prior knowledge of the form of the model output, and ideally construct algorithms to automatically extract these features from the output data.

An alternative approach to sensitivity analysis of dynamic model output has been suggested by Campbell et al. (2006). For a set of N model evaluations, the output of each model run is treated as a function of time, $y_i(t)$, which can be expanded using an appropriate set of basis functions $\Phi(t) = (\phi_1(t), \phi_2(t), ..., \phi_m(t))$ such that:

$$y_i(t) = \sum_{k=1}^{m} \omega_{ik}\phi_k(t) \quad \text{for } i = 1, ..., N \tag{4.1}$$

The basis functions, $\phi_k(t)$, represent different aspects of the functional model output. The scalar coefficients of the expansion, ω_{ik}, indicate how much of each basis function is contained in each model run.

Sensitivity analysis techniques can be applied to the coefficients of the expansion to investigate their dependence on the model parameters. If the coefficients of a given basis function $\mathbf{\Omega}_k = (\omega_{1k}, \omega_{2k}, ..., \omega_{Nk})$ are sensitive to a parameter, then that parameter is important in producing the type of variation in the model output which is described by the corresponding basis function, ϕ_k. This approach was demonstrated by Campbell et al. (2006) on a simple example using a graphical method to perform the sensitivity analysis.

The use of a basis set expansion, when coupled with more advanced global SA techniques, could potentially be used to study the sensitivity of dynamic biological models. The development of this method is presented in this chapter beginning with a discussion of the use of principal component analysis (PCA) as a way to expand the functional model outputs.

4.2 Basis Set Expansion of Functional Data

The representation of data using a set of basis functions is an important step in most functional data analysis techniques (Ramsay and Silverman, 2002). There are a number of standard pre-defined basis sets that can be used to represent a set of functional model outputs. However, a particular basis set will generally only be suitable for certain types of data (Campbell et al., 2006). The choice of an appropriate basis is therefore problem specific much like the task of defining a set of scalar features from the model output.

An alternative to using a pre-defined basis set is to determine the basis functions from the data. The use of data-driven basis functions is largely problem-independent. Data-driven basis functions are also often more interpretable in physical terms and typically capture the important variation in the output in a smaller number of functions than pre-defined bases (Campbell et al., 2006). The main strength of a data-driven approach is that the aspects of the output described by the basis functions are based on the important types of variation in the data and not prior notions of what we believe is of interest. This ability to automatically extract the features from the data is particularly attractive as it avoids the risk of "blinding ourselves to important differences by what we choose to look for" (Jones and Rice, 1992). One example of a data driven basis set are principal components calculated via a principal component analysis (PCA).

4.2.1 Principal Component Analysis

PCA is a multivariate statistical procedure which seeks to reduce the dimensionality of a data set made up of a larger number of interrelated variables while maintaining as much of the variation in the data as possible (Jolliffe, 2002). The original data is transformed into a new set of variables known as the principal components (PCs) which are uncorrelated and arranged such that successive PCs contain decreasing amounts of the variation present in the original data.

For a set of N observations of q variables, $\mathbf{y}$, PCA can be seen as finding the q weight vectors $\xi_z = (\xi_{1z}, ..., \xi_{qz})^{'}$, $z = 1, ..., q$ for which the linear combinations:

$$f_{iz} = \sum_{j=1}^{q} \xi_{jz} y_{ij} \tag{4.2}$$

have the largest possible variance described by the mean square:

$$\frac{1}{N} \sum_{i=1}^{N} f_{iz}^2 \tag{4.3}$$

subject to the constraints:

$$\sum_{j=1}^{q} \xi_{jz} = 1 \quad \text{for } z = 1, ..., q \tag{4.4}$$

$$\sum_{j=1}^{q} \xi_{jk} \xi_{jm} = 0 \quad k < m \tag{4.5}$$

The first constraint ensures that the problem is well defined and that the sum of squares can not be arbitrarily large. The second constraint ensures that the second and subsequent weight vectors are orthogonal and hence unrelated to the previous ones. It is also important to subtract the mean

from the data before performing PCA. This ensures that maximising the mean square of the f_{iz} is equivalent to maximising their variance. The amount of variation described by the PCs declines as z increases. Therefore typically only a subset of the principal components, $q_s << q$ will be of interest.

In the case of functional data, the variable values are replaced by functions $y_i(t)$ and PCA is equivalent to finding the linear combinations of weight functions $\beta_z(t)$ which maximise the variation in the f_{iz}s subject to continuous versions of 4.4 and 4.5.

$$f_{iz} = \int \beta_z(t) y_i(t) dt \tag{4.6}$$

The weight functions are the principal components of the data and constitute a set of basis functions which represent the different types of variation within the data. The importance of the different types of variation is measured by the fraction of the variance in the original data accounted for by each PC. The principal component scores, f_{iz}, are the coefficients of the expansion of the original data using the PCs as basis functions.

The use of PCA to investigate collections of curves was previously suggested by Jones and Rice (1992) as part of a method to display their important features and the technique is regarded as a key tool in functional data analysis (Ramsay and Silverman, 1997).

4.2.2 Calculating Functional Principal Components

The calculation of principal components is generally defined in terms of the eigenanalysis of the covariance matrix of the $N \times q$ matrix containing the mean centred data. The eigenvectors are the principal components of the data and the eigenvalues describe the distribution of the total variance. Details can be found in a number of textbooks (see for example Jolliffe, 2002) and standard computer algorithms exist for the solution of the eigenequation.

In the case of functional data principal components can also be viewed in terms of an eigenanalysis of the covariance function (Ramsay and Silverman, 1997). There are two approaches to the calculation of functional principal components. These are described briefly below.

The first approach to calculating the principal components of functional data is to discretize the N functions on some regular grid of time points. This data can be treated as a set of N observations of T variables (where T is the number of time points at which the model is evaluated). This $N \times T$ matrix can then be passed to a standard PCA algorithm (e.g. `R`'s princomp routine) to calculate the principal components and the associated scores. Using this method the maximum number of principal components, q, is equivalent to the number of time points (i.e the number of variables).

Models will typically be solved using some numerical method so the output will already be in the form of discrete time-value pairs. The major problem with this approach is that the time points must be evenly spaced. This may not be the case if the model is solved using a method with an adaptive step size.

The second method for calculating the principal components of functional data requires the data to first be expanded using some pre-defined basis set. The principal component analysis can then be defined as an eigenanalysis problem in terms of the covariance of the coefficients of the expansion (Ramsay and Silverman, 1997).

Software for the calculation of the principal components using this approach is provided in the `fda` package (Ramsay et al., 2008) for the statistical programming language `R` (R Development Core Team, 2008). The number of principal components, q, which can be calculated by this approach is equal to the number of basis functions used in the initial expansion of the data.

The basis function approach is more flexible than the discretization approach. It does not require the time points to be the same for each model run. Therefore this approach will be used in the rest of this thesis.

4.2.3 Functional PCA of Model Outputs

The detailed insulin signalling pathway model described in chapter 3 is used to demonstrate the use of functional PCA to extract the interesting modes of variation from a set of dynamic model outputs.

The insulin model contains 21 parameters which have been extracted or derived from the literature (see table 3.3 and section 3.5.2). These parameters may have a certain amount of uncertainty or imprecision associated with them. Even if the parameters are known precisely we may wish to investigate how perturbing them influences the system behaviour.

For the purposes of demonstrating the technique each parameter is assumed to vary uniformly in a range of $\pm 50\%$ of its nominal value (see table 3.3). The uncertain parameters represent a 21-dimensional input space. A sample of 1000 input vectors was generated from this space using the quasi-random Sobol sequence. The model was then evaluated for each input sample to produce a set of 1000 model outputs.

The external input to the model was chosen to represent a constant concentration of insulin of magnitude $1 \times 10^{-6} M$, from $t = 0$ until $t = 30$ minutes after which the ligand is removed. The model is run until $t = 60$ minutes to allow the dephosphorylation of GSK3 to be studied. The model is solved in XPPAUT (Ermentrout, 2002). Figure 4.1 shows the set of model outputs generated by the 1000 run sample.

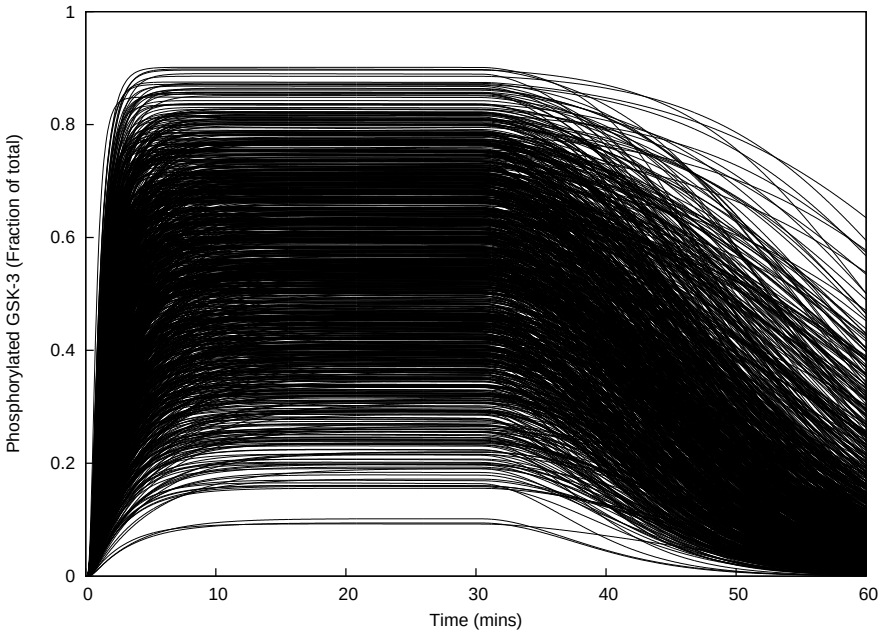


Figure 4.1: The output from a sample of 1000 evaluations of the insulin model.

This plot shows the typical shape of the model output in response to the chosen input function. It also shows that there is considerable variation in the output due to the uncertainty in the model parameters. The range of this variation is shown by the envelope which encloses all the curves. However information about the behaviour of individual curves and the types of variation present in the set is not clear from this type of display. Functional principal component analysis can be used to extract this information.

The PCs of the insulin model output were calculated using the basis set method, implemented in `R` via the `fda` package (Ramsay et al., 2008). Principal components calculated via the discretization approach were identical. The left hand panels of figure 4.2 show the first three PCs for the output of the insulin model.

The first principal component accounts for 90.6% of the variation in the model output indicating that this is the dominant mode of variation found in the data. The first PC is positive throughout the entire time course but places considerably more weight on times between $\sim$ 5-40 minutes, the period in which the model output is typically in a steady state. This describes a vertical shift in the time-course of inactive GSK3 with the greatest increase in the steady state concentration. Model runs which have high positive scores for the first PC will have higher than average values across all time points and significantly higher steady state values. Negative scores imply a reduced concentration of inactive GSK3.

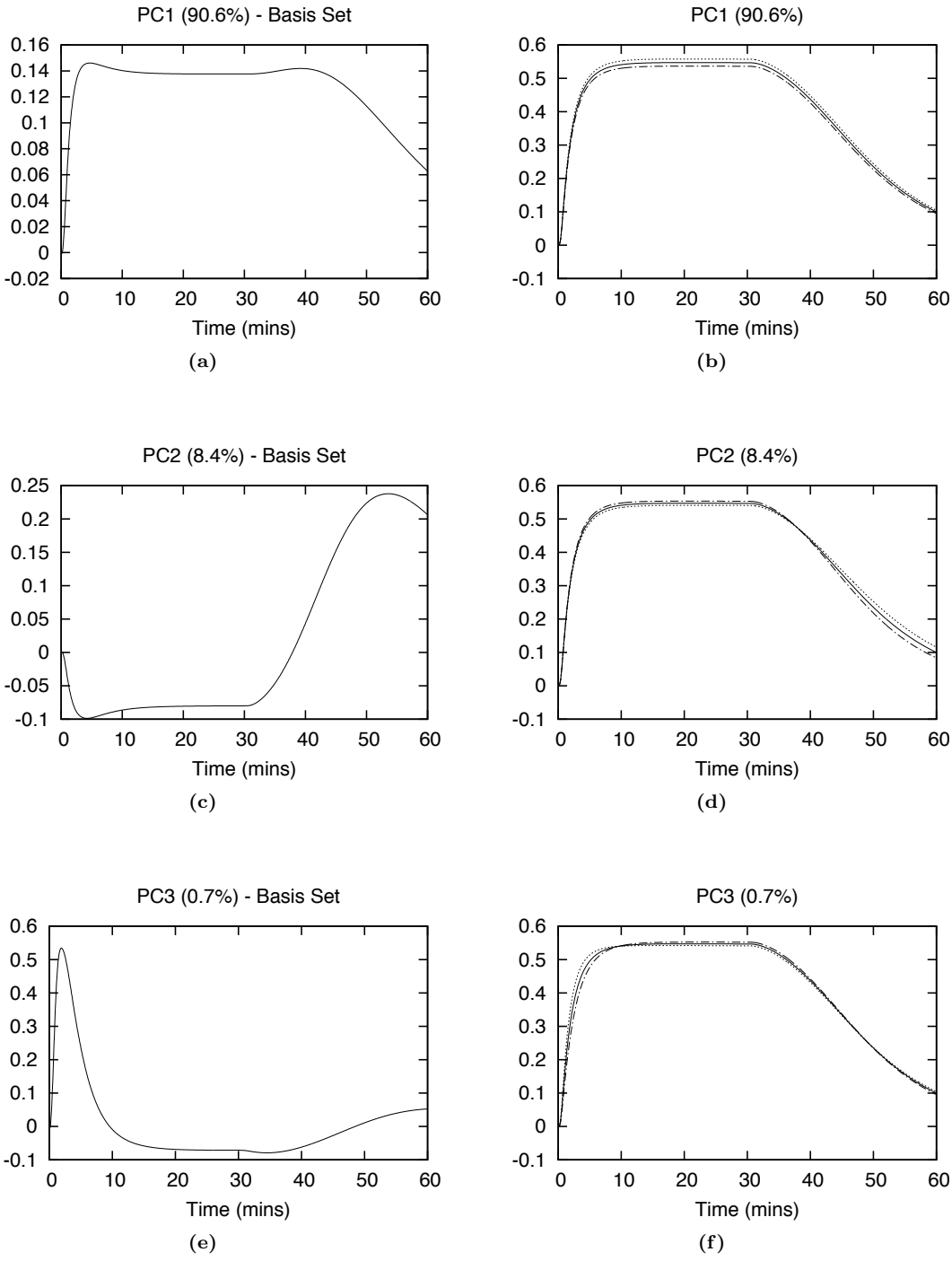


Figure 4.2: The first 3 principal components of the insulin model output. The principal components were calculated using a b-spline basis set. Panels a,c, and e show the PC curves. Panels b,d, and f show the mean model output (solid line) plus (dotted line) and minus (dashed line) a multiple of the principal components. The percentages show the amount of the total variation described by each component. Together the first 3 principal components describe 99.7% of the variation in the model output.

The second PC accounts for a much smaller amount of variation, 8.4%. It places a negative weight on times up to ∼40 minutes and a large positive weight on the subsequent part of the time-course, after the stimulus is removed. Positive scores on this PC produce a small reduction in the steady state concentration and an increase in the amount of inactive GSK3 present at later times. This corresponds to a decrease in the rate at which GSK3 is de-phosphorylated after insulin is removed from the system.

The third PC (0.7% of the total variation) places large positive weights on the early time points. This period corresponds to the inactivation phase of GSK3. Positive scores on this component will be found for model runs which have an increased rate of inactivation.

The interpretation of the PCs from plots of the components themselves is not always so straightforward (Ramsay and Silverman, 1997). A clearer picture can often be obtained by plotting the mean function of the output sample plus and minus some multiple of the PCs. This approach is demonstrated in the right hand panels of figure 4.2. The main effect of each PC, as described above, is clearly shown in these plots: PC1 describes the variation in the steady state concentration, PC2 describes the variation in the rate at which GSK3 is dephosphorylated and PC3 the variation in the inactivation process.

The multiple of the principal components to use in the construction of plots like figure 4.2 is largely subjective and can be modified to produce interpretable results. Ramsay and Silverman (1997) suggest plotting the mean, $\hat{\mu} \pm 0.2CPC_z$ where C is the root-mean-square difference between $\hat{\mu}$ and its time average, $\bar{\mu}$:

$$C^2 = T^{-1}\|\hat{\mu} - \bar{\mu}\|^2 \tag{4.7}$$

$$\bar{\mu} = T^{-1}\int \hat{\mu}(t)dt \tag{4.8}$$

and the factor of 0.2 was chosen to give useful results. This approach was followed in figure 4.2 where a value of 0.5 was used to best illustrate the modes of variation.

This section has demonstrated how functional PCA can be used to extract the main modes of variation from a set of output curves generated from a mathematical model. These modes of variation can be interpreted in terms of specific aspects or features of the model output. Importantly the fraction of the total variation in the model output described by each mode decreases rapidly with the order of the principal components. It is therefore only necessary to consider a small number of the components to describe almost all the variation in the model output.

The second part of the proposed method is to study how these modes of variation depend on

the model inputs. This is achieved by using the scalar PC scores as the "output" of the model in the sensitivity analysis. The following section discusses the use of variance based SA techniques to do this.

4.3 Variance Based Sensitivity Analysis

Variance based methods are a class of global sensitivity analysis techniques which "estimate how much output variability is dependent on each of the input factors (taken singly and in combination with one another)" (Archer et al., 1997). The importance of a (set of) factor(s) is based on how much it (they) control the model prediction (Saltelli et al., 2000a) and is measured by the reduction in the output variance obtained by "fixing" those factors (Homma and Saltelli, 1996). Variance based methods are considered by many to be the best SA techniques for a wide range of scenarios due to their model independence and their ability to quantitatively assess the impact of the model inputs. The basic concept of this class of methods is illustrated below for a general model of the form:

$$Y = f(\mathbf{X}) \tag{4.9}$$

where $\mathbf{X} = (x_1, x_2, ..., x_k)$ is a k-vector of uncertain model factors.

If all the factors are allowed to vary over their entire range of values then the uncertainty in the model output Y can be quantified by its unconditional variance $V_{\mathbf{X}}(Y)$. The question addressed by variance based methods is: How does removing the uncertainty in factor x_i (i.e. fixing it at its true value) reduce the variance in the model output Y? If the reduction in the variance achieved by fixing x_i is large then x_i is an important factor in determining the variation in the model output.

The effect on the variance of fixing x_i is given by the conditional variance:

$$V_{\mathbf{X}_{-i}}(Y|x_i = x_i^*) \tag{4.10}$$

The notation $\mathbf{X}_{-i}$ indicates that all other factors in $\mathbf{X}$ are allowed to vary. The true value of x_i^* is not known so the conditional variance is averaged over all possible values of x_i:

$$E_{x_i}(V_{\mathbf{X}_{-i}}(Y|x_i)) \tag{4.11}$$

(In future the subscript notation on the expected value and variance will be dropped for simplicity so that in $E(V(Y|x_i))$, E is understood to be over x_i and V over $\mathbf{X}_{-i}$.)
The smaller the value of $E(V(Y|x_i))$ the larger the influence of x_i on the model output and the

more important the factor. The total variance is a constant and can be expressed using the "*law of total variance*" as:

$$V(Y) = V(E(Y|x_i)) + E(V(Y|x_i)) \tag{4.12}$$

Therefore, selecting factors with small $E(V(Y|x_i))$ as important is equivalent to selecting those with high values of $V_i = V(E(Y|x_i))$, also known as the variance of the conditional expectation (VCE). Various variance based sensitivity measures, and schemes to estimate them, have been suggested in the literature. McKay (1995) defined the correlation ratio, η^2 as the ratio of the VCE to the total variance while Hora and Iman (1986) used the square root of the VCE as a measure of the importance of factor x_i.

Both these measures can be shown to be equivalent to the first order sensitivity indices of the Sobol method (Sobol, 1993), a Monte Carlo variance-based method. The Sobol method is an efficient technique for the calculation of the individual factor importance measures and also allows investigation of higher order effects, i.e. the influence of interactions between input factors. An overview of the Sobol method is provided in the following section.

4.3.1 The Method of Sobol

The method of Sobol (Sobol, 1993) is based on a decomposition of the model output $Y = f(\mathbf{X})$ into terms of increasing dimensionality. The function $f(\mathbf{X})$ can be written as the sum:

$$f(\mathbf{X}) = f_0 + \sum_{i=1}^{k} f_i(x_i) + \sum_{1 \le i < j \le k} f_{ij}(x_i, x_j) + ... + f_{1,2,...,k}(x_1, ..., x_k) \tag{4.13}$$

provided that f_0 is a constant and the integral of every term over any of its variables is zero. A consequence of this decomposition is that:

$$f_0 = \int_{\Omega^k} f(\mathbf{X}) d\mathbf{X} \tag{4.14}$$

The total variance of $f(\mathbf{X})$ can be written as:

$$V = \int_{\Omega^k} f^2(\mathbf{X}) d\mathbf{X} - f_0^2 \tag{4.15}$$

This can also be decomposed in the same manner as the function itself:

$$V = \sum_{i=1}^{k} V_i + \sum_{1 \le i < j \le k} V_{ij} + ... + V_{12...k} \tag{4.16}$$

The terms of this decomposition are the contributions to the variance from term $f_{i_1...i_s}$ in (4.13) and are given by:

$$V_{i_1...i_s} = \int_0^1 ... \int_0^1 f^2_{i_1...i_s}(x_{i_1}, ..., x_{i_s}) dx_{i_1} ... dx_{i_s} \tag{4.17}$$

The importance measures or Sobol indices are then defined as:

$$S_{i_1..i_s} = \frac{V_{i_1..i_s}}{V} \tag{4.18}$$

The term $S_{i_1..i_s}$ gives the fraction of the total variance which is due to any individual factor or combination of factors. For example, $S_i = V_i/V$, called the first-order sensitivity index, is the contribution of x_i to the output variation. S_{ij} for $i \neq j$ is that part of the variation due to x_i and x_j which is not explained by the sum of the first-order effects of x_i and x_j. This is the variance which is due to the interaction between those factors.

The key to the Sobol method is that the integrals in (4.14) (4.15) and (4.17) can be evaluated using Monte Carlo integrals. For an input sample of size N with k-d elements $\mathbf{X}_m$, each of which is a model input vector:

$$\hat{f}_0 = \frac{1}{N} \sum_{m=1}^{N} f(\mathbf{X}_m) \tag{4.19}$$

$$\hat{V} = \frac{1}{N} \sum_{m=1}^{N} f^2(\mathbf{X}_m) - \hat{f}_0^2 \tag{4.20}$$

The first order effects require estimates for the V_is which are given by equation 4.21 (for a derivation see Homma and Saltelli (1996)):

$$\hat{V}_i = \frac{1}{N} \sum_{m=1}^{N} f(\mathbf{X}^{(1)}_m) f(\mathbf{X}^{(2)}_{(\sim i)m}, x^{(1)}_{im}) - \hat{f}_0^2 \tag{4.21}$$

where the superscripts (1) and (2) refer to two different input samples. To calculate the $\hat{V}_i$s we

multiply values of the model output calculated using the first sample by values calculated using the second sample, but with factor x_i taken from the first sample. In other words, we "resample" all factors except the factor of interest x_i. Intuitively we can see that if x_i is an important factor, high values of $f(\mathbf{X}_m^{(1)})$ will be multiplied by high values of $f(\mathbf{X}_{(\sim i)m}^{(2)}, x_{im}^{(1)})$ and low values by low values resulting in a high value for $\hat{V}_i$. If x_i is not influential high and low values of the two terms will be paired randomly and $\hat{V}_i$ will be lower. The computational cost associated with calculating a complete set of first order effects is $N(k+1)$ model evaluations, one sample of size N to calculate $\hat{f}_0$ and $\hat{V}$ and k samples of size N, in which factor $i = 1, ...k$ is kept the same. The accuracy of the estimates provided by the Monte-Carlo integrals will increase as the sample size, N, is increased. Unfortunately there is no way to know what a sufficient value of N will be before conducting the analysis. It is therefore necessary to check the convergence of the indices as the sample size is increased. Typically N will be of the order of a few hundred to a few thousand to obtain satisfactory convergence of the indices.

The second order effects require estimates for V_{ij} which are given by (Homma and Saltelli, 1996):

$$\hat{V}_{ij} = \frac{1}{N}\sum_{m=1}^{N} f(\mathbf{X}_m^{(1)}) f(\mathbf{X}_{(\sim ij)m}^{(2)}, x_{im}^{(1)}, x_{jm}^{(1)}) - \hat{f}_0^2 - \hat{V}_i - \hat{V}_j \tag{4.22}$$

where all factors except x_i and x_j are resampled in the second term in the product. Similar expressions can be derived for the higher order terms.

It can be seen from the definitions given above that the calculation of each effect requires the evaluation of the model for an additional sample of size N. The decomposition in equation 4.16 contains $2^k - 1$ terms, therefore the total cost of evaluating all effects is $N2^k$. This is not practical unless k is small.

Total Effect Indices

As an alternative to calculating the entire set of indices, Homma and Saltelli (1996) suggested a single measure which captures the total effect of a factor on the model output. The measure is based on the idea introduced by Sobol (1993) of partitioning the factors into two subsets. If we define one set to contain only factor x_i and the other set $\mathbf{X}_{\sim i}$ contains all other factors then the total variance can be written as:

$$V = V_i + V_{\sim i} + V_{i,\sim i} \tag{4.23}$$

and the total effect of x_i on the output is:

$$V_i^{tot} = V_i + V_{i,\sim i} = V - V_{\sim i} \tag{4.24}$$

The total effect index is defined as:

$$S_{Ti} = \frac{V_i^{tot}}{V} = 1 - \frac{V_{\sim i}}{V} \tag{4.25}$$

and describes the total variance accounted for by factor i individually and in all possible interactions with other parameters. Saltelli (2002) showed that the total effects can be calculated together with the first order effects for the extra cost of N model runs using equation 4.21 to estimate $V_{\sim i}$:

$$\hat{V}_{\sim i} = \frac{1}{N} \sum_{m=1}^{N} f(\mathbf{X}_m^{(2)}) f(\mathbf{X}_{(\sim i)m}^{(2)}, x_{im}^{(1)}) - \hat{f}_0^2 \tag{4.26}$$

The set of S_is and S_{Ti}s provide an efficient way to quantify the importance of individual inputs and interaction terms at the expense of information about specific interactions.

The Sobol indices have a number of useful properties. Firstly the sum of the sensitivities of all orders is always equal to 1 and the sum of the first order effects S_i will be ≤ 1. The difference between $\sum_i S_i$ and unity provides a measure of the amount of variance which is accounted for by interactions. Similarly, for any parameter i, the difference between S_i and S_{Ti} indicates the extent to which it is involved in interactions. The sum of the S_{Ti}s will typically be larger than 1 because interactions are counted multiple times.

Computing the Sobol Indices

The computation of the first order and total effect Sobol indices can be carried out using the following steps:

1. Generate two N by k random input matrices $X^{(1)}$ and $X^{(2)}$
2. Construct k, N by k "resample" matrices $X^{(2)}_{(\sim i)}, x^{(1)}_i$ in which the i^{th} column is taken from $X^{(1)}$
3. Solve the model for each of the $N(k+2)$ input vectors
4. Calculate estimates for f_0 and V using equations 4.19 and 4.20
5. Calculate estimates for V_i and $V_{\sim i}$ using equations 4.21 and 4.26 for each parameter $i = 1, ..., k$
6. Calculate the S_is and S_{Ti}s

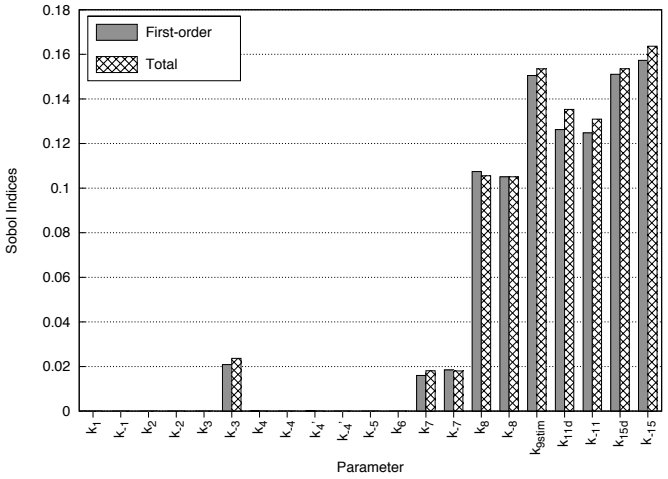
4.3.2 Principal Component Based Sobol Indices

In section 4.2.3 functional PCA was used to extract the key modes of variation from a Monte-Carlo output of the insulin model. This section shows how the Sobol method can be used to investigate how these modes of variation depend on the parameter values by applying the method to the PC scores.

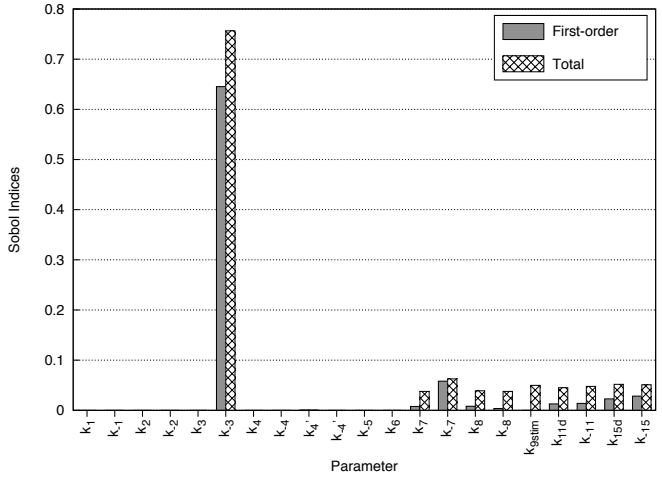
The application of the Sobol method to the principal components requires an additional step in the computational algorithm outlined above. After evaluating the model for each input vector functional PCA is used to calculate the principal components of the set of $N(k+2)$ model outputs. Steps 4-6 are then carried out for each principal component using the PC scores as the model "output". This gives us a set of first order and total effect indices S^z_i and S^z_{Ti} for each PC $z = 1, ...q$.

Using the same parameter ranges and input function described in section 4.2.3 the PCA Sobol method was applied to the insulin model. A sample size $N = 2000$ was used to ensure convergence of the indices. The PCs calculated from the Sobol output are the same as those shown in figure 4.2 as were the fractional variances they describe. The first order and total effect indices are shown in figure 4.3 for the first three PCs.

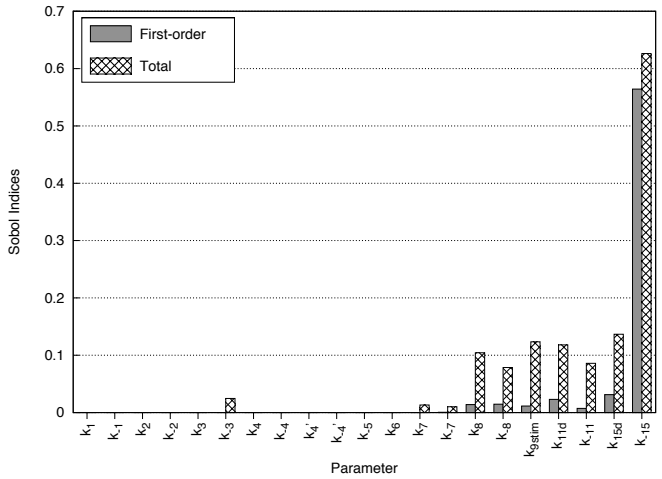
Only 10 parameters have significantly non-zero effects on the model output. These are all involved in the post receptor signalling pathway with the exception of k_{-3} the rate of insulin dissociation. The parameters of the receptor recycling subsystem have no effect on the model



(a) PC1



(b) PC2



(c) PC3

Figure 4.3: The first order and total effect Sobol indices of the insulin model. The three plots show the indices for the scores of the first three principal components.

output. This would appear to agree with the view that post insulin receptor defects represent the primary sites leading to insulin resistance (Pessin and Saltiel, 2000).

The first PC primarily affects the maximal phosphorylation of GSK3. The conversion of PI(4,5)P2 to PI(3,4,5)P3 (k_{9stim}) and the de/phosphorylation of GSK3 (k_{-15} and k_{15d}) are found to be particularly important in producing variation in the scores on this component. The lesser importance of PI3K activation and inactivation, k_8 and k_{-8}, supports the experimental observations that reduced insulin stimulated activation of PI3K does not affect the downstream activation of Akt (Kim et al., 1999). There is little interaction between parameters as indicated by the minimal differences between the first order and total effects for individual parameters and the sum of the first order indices, $\sum_i S_i^1 = 0.97$.

The uncertainty in the second PC score is dominated by k_{-3} which accounts for 65% of the variation in this component. k_{-3} describes the deactivation of insulin receptors resulting from dissociation of insulin and dephosphorylation of the receptor by PTPs. This explains its importance in controlling the reactivation of GSK3 following removal of the external insulin input (the behaviour described by the second PC). This result is also consistent with experimental evidence that insulin signalling can be enhanced by reducing the activity of PTPs (Goldstein et al., 1998) and that PTPs represent potential therapeutic targets for the management of insulin resistance (Drake and Posner, 1998). Figure 4.3 also shows that there is an increased role of interactions in the second PC ($\sum_i S_i^2 = 0.74$). This is particularly evident in the case of k_{-3} where the difference between the total and first order indices is 0.11.

The third PC (which describes the initial phosphorylation of GSK3) is largely controlled by k_{-15}, the dephosphorylation rate of the kinase. This is in line with the view that processes downstream of Akt are crucial in propagating the insulin signal (Brady and Saltiel, 1999). As with the second PC there is a significant interaction effect, especially for the parameters k_8, k_{9stim}, k_{11d} and k_{15d}. The importance of interactions highlights the need to use global SA methods to understand the behaviour of biological systems. Local methods, in which parameters are varied one at a time do not allow the possible effects of interactions between parameters to be explored.

This section has shown how the Sobol method can be used to quantify the effects of the model parameters on the modes of variation described by the principal components. It is also of interest to know which parameters are most important in terms of their effect on the entire model output. This information is given by the overall sensitivity indices, S_i^O and S_{Ti}^O defined in the next section.

4.3.3 The Overall Sensitivity Index

The PC based Sobol indices S_i^z give the fraction of the variance in the z^{th} PC score which is due to the i^{th} parameter. If we define V_{PC}^z to be the fraction of the total variance described by the z^{th} PC then:

$$S_i^O = \sum_{z=1}^{q} S_i^z V_{PC}^z \tag{4.27}$$

quantifies the fraction of the total output variance due to the individual effects of parameter i. This is a measure of the overall first order effect of parameter i on the model output. Similarly the overall total effect of parameter i (including its interactions with other parameters) is given by:

$$S_{Ti}^O = \sum_{z=1}^{q} S_{Ti}^z V_{PC}^z \tag{4.28}$$

Because the variance described by successive PCs decreases rapidly it is not necessary to include all the principal components, $z = 1, ..., q$, in the summations in equations 4.27 and 4.28. A good approximation of the overall sensitivities can be obtained by using a subset, q_s, of the PCs.

Table 4.1 contains the overall first and total order sensitivities for the insulin model calculated using 1,2,3 and 4 PCs. The parameters are listed in order of increasing importance (for $q_s = 2$). The results show that increasing the number of PCs beyond $q_s = 2$ has a minor effect on the quantitative values of the sensitivities and importantly makes no difference to the importance ranking of the parameters.

	S_i^O				S_{Ti}^O			
Parameter	$q_s = 1$	$q_s = 2$	$q_s = 3$	$q_s = 4$	$q_s = 1$	$q_s = 2$	$q_s = 3$	$q_s = 4$
k_{-1}	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
k_{-2}	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
k_3	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
$k_{-4}^{'}$	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
k_{-5}	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
k_6	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
k_1	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
k_2	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
k_4	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
k_{-4}	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
$k_4^{'}$	0.00011	0.00016	0.00016	0.00016	0.00011	0.00016	0.00016	0.00016
k_7	0.01446	0.01511	0.01511	0.01542	0.01641	0.01958	0.01967	0.02006
k_{-7}	0.01671	0.02160	0.02160	0.02188	0.01680	0.02162	0.02163	0.02215
k_{-3}	0.01890	0.07310	0.07310	0.07378	0.02141	0.08496	0.08513	0.08682
k_{-8}	0.09520	0.09553	0.09562	0.09565	0.09525	0.09836	0.09891	0.09911
k_8	0.09732	0.09800	0.09810	0.09810	0.09761	0.09889	0.09962	0.09982
k_{-11}	0.11304	0.11420	0.11425	0.11425	0.11865	0.12267	0.12327	0.12346
k_{11d}	0.11440	0.11548	0.11564	0.11565	0.12259	0.12638	0.12721	0.12739
k_{9stim}	0.13634	0.13634	0.13642	0.13642	0.13906	0.14325	0.14411	0.14429
k_{15d}	0.13687	0.13879	0.13901	0.13901	0.13910	0.14346	0.14441	0.14459
k_{-15}	0.14253	0.14492	0.14887	0.14887	0.14827	0.15256	0.15694	0.15726

Table 4.1: The first and total order overall sensitivity indices of the insulin model. The overall indices measure the sensitivity of the entire model output to the model parameters. The values are calculated using 1, 2, 3 and 4 PCs. The inclusion of higher order PCs has a minimal effect on the quantitative values of the indices. For $q_s > 2$ no change is observed in the ranking of the parameters by the overall sensitivities.

4.3.4 Time Varying Sobol Indices

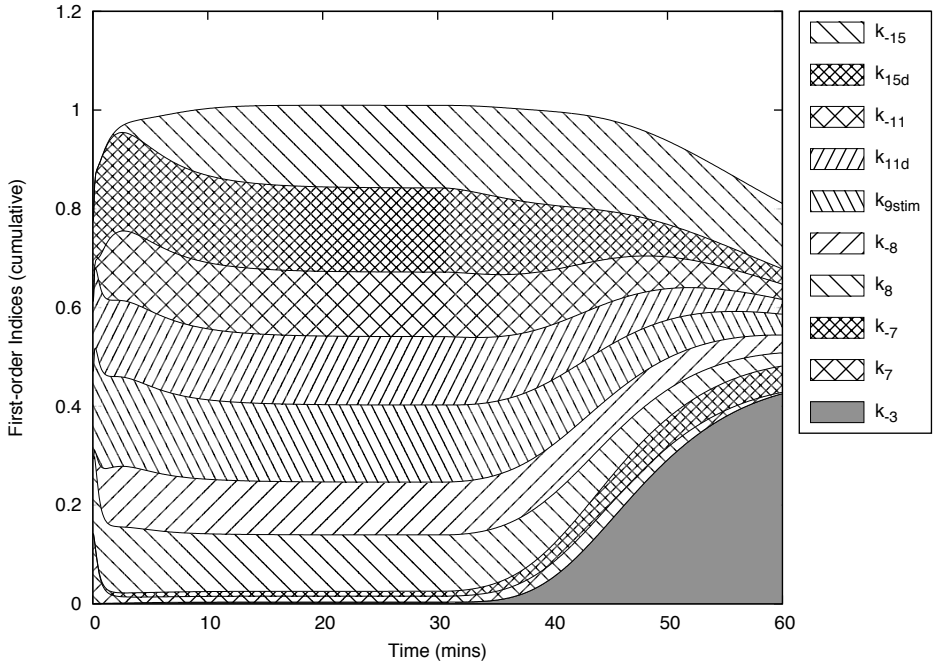
The usual approach to sensitivity analysis of time-dependent model output is to calculate the sensitivities at each discrete time point at which the model is evaluated. The time-varying sensitivities of the insulin model have been calculated for comparison with the PC based results. The indices are displayed as cumulative area plots. Such plots allow the information on multiple parameters to be displayed in a single figure and provide a visual representation of the total amount of variance explained by the first order effects.

Figure 4.4 shows how the first and total effect Sobol indices vary over time in the insulin model. The ten most influential parameters are displayed, the indices for other parameters being zero or close to zero. These are the same parameters identified as influential by the PCA based method. It can be seen that there are three distinct phases in the sensitivity profiles. These correspond to the first three principal components.

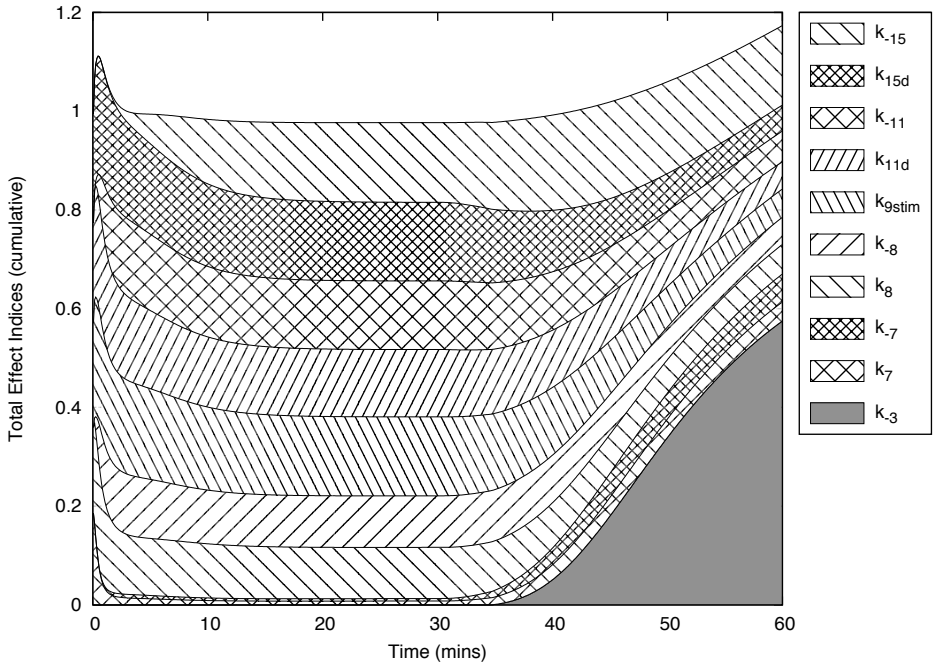
Between 0 and 10 minutes the model output describes the initial phosphorylation of GSK3. This phase corresponds to the third PC. Like the PC based indices the variation in this phase involves

interactions between the model parameters. However, unlike the PC indices, the sensitivity is more evenly distributed among the parameters of the post receptor signalling pathway with less importance ascribed to k_{-15}. From $\sim$ 10 minutes to $\sim$ 35 minutes the model output is in steady state, as described by PC1. The variation in this phase is almost completely described by individual effects and dominated by the processes in the post receptor signalling pathway. From $\sim$ 35 minutes onwards the importance of k_{-3} increases significantly, accounting for $\sim$ 40% of the variance at $t = 60$ minutes. This is consistent with the results for the second principal component. There is also an increase in the interaction effects during this phase.

In the case of the insulin model the time-varying sensitivities are relatively easy to interpret and provide similar information about the model behaviour as the PC based indices. However, the model output is quite simple and the behaviour is clearly divided into three phases which correspond to the first three principal components. For more complicated functional output this is not necessarily the case and it can be difficult to extract information about different aspects of the output. This is shown by the analysis of the glucagon receptor model presented in the next section.



(a)



(b)

Figure 4.4: The time varying Sobol indices of the insulin model. Panel a shows the first order indices, panel b shows the total effects. The indices are displayed as cumulative area plots.

We can also obtain a measure of the effect of a parameter on the complete model output by calculating the average of the time-varying sensitivity indices (first order or total) over all time points.

$$S_i^{Avg} = \frac{1}{T}\sum_{j=1}^{T} S_i^j \tag{4.29}$$

where S_i^j is the sensitivity of the model output at time point t_j to parameter i and T is the number of time points. Figure 4.5 shows the results for the insulin model. The ranking obtained using S^{Avg} is the same as that given by S^O.

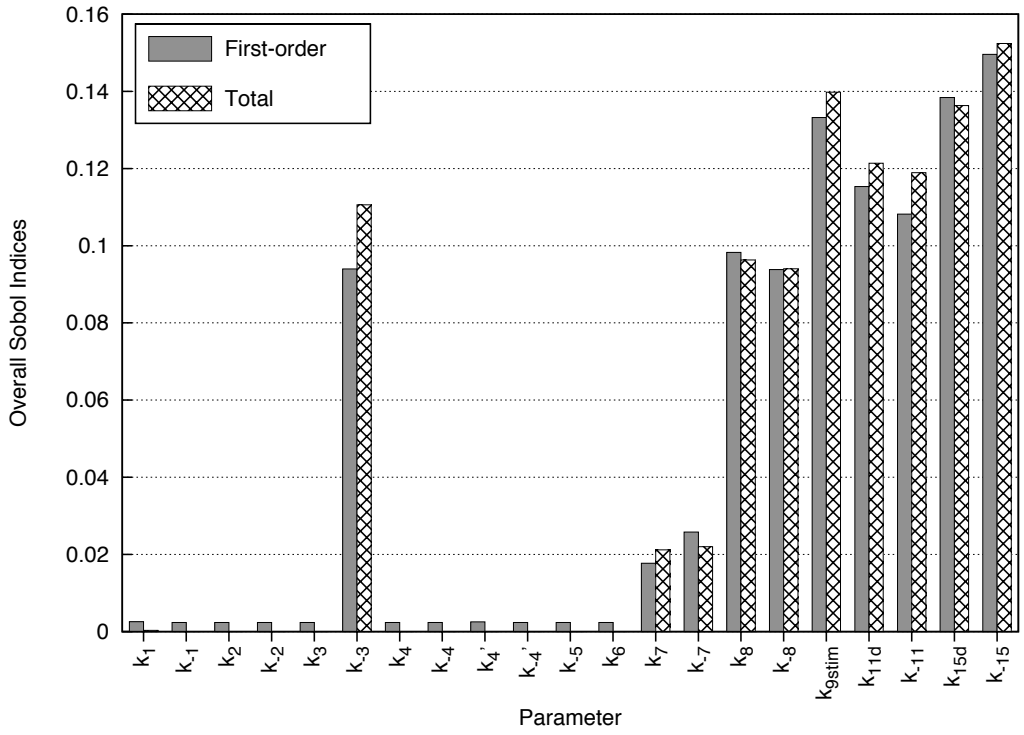


Figure 4.5: The first order and total effect Sobol indices of the insulin model based on a time average.

The problem with this measure is that, unlike the overall sensitivity indices (equations 4.27 and 4.28) in which the components of the sum are weighted by the amount of variance they describe, each time-point is given equal weight in the summation in equation 4.29. This has the potential to incorrectly identify as important parameters which have little effect on the overall variation in the model output. This is demonstrated by the application to the glucagon receptor model presented below.

As a final point, it is worth noting that the time-varying and PCA based sensitivities can be

obtained from the same set of model evaluations. As the main expense of the variance based methods is in the evaluation of the model it is possible to calculate both sets of indices to compare the information they provide at little additional computational cost.

4.3.5 Application of the Sobol Method to the Glucagon Receptor Model

This section demonstrates the application of the approach developed above to the glucagon receptor model. The model (see section 3.3.2) describes the activation of PLC by glucagon via G-protein coupled receptors. The behaviour of the model at its nominal parameter point in response to a repeated glucagon stimulus is shown in figure 4.6. The model displays a reduced response to the second stimulus due to desensitisation of the receptor.

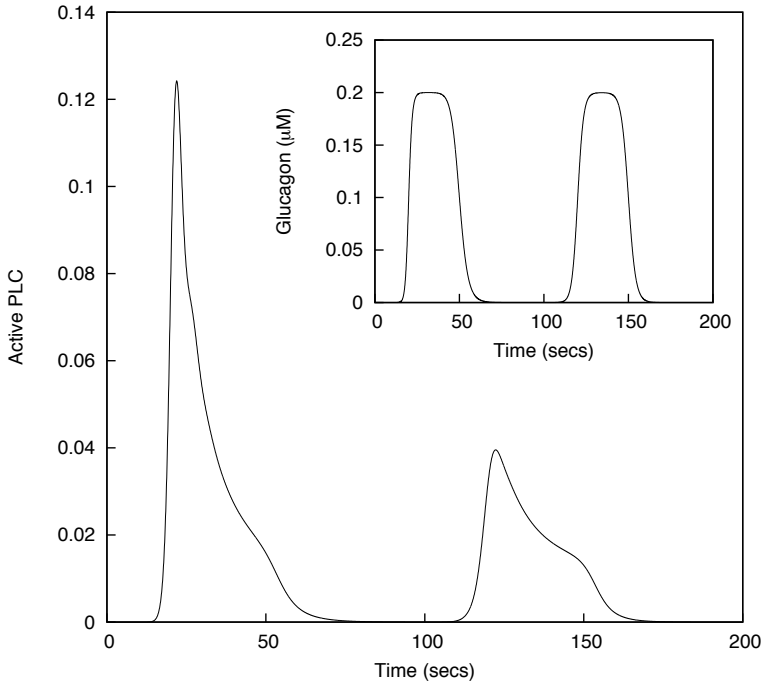


Figure 4.6: The output of the glucagon receptor model at its nominal parameter values in response to a repeated glucagon stimulus (inset).

Principal Component Sobol Indices

The method was applied to the model using the repeated glucagon stimulus as an external input. For the purposes of an initial exploration uniform distributions for each parameter in the range ±50% of the nominal values were used. Figure 4.7 shows the first three PCs of the model output together with their effects on the mean timecourse. The first three components capture 98.2% of the output variance.

The principal components are less easy to interpret than those of the insulin model. The first

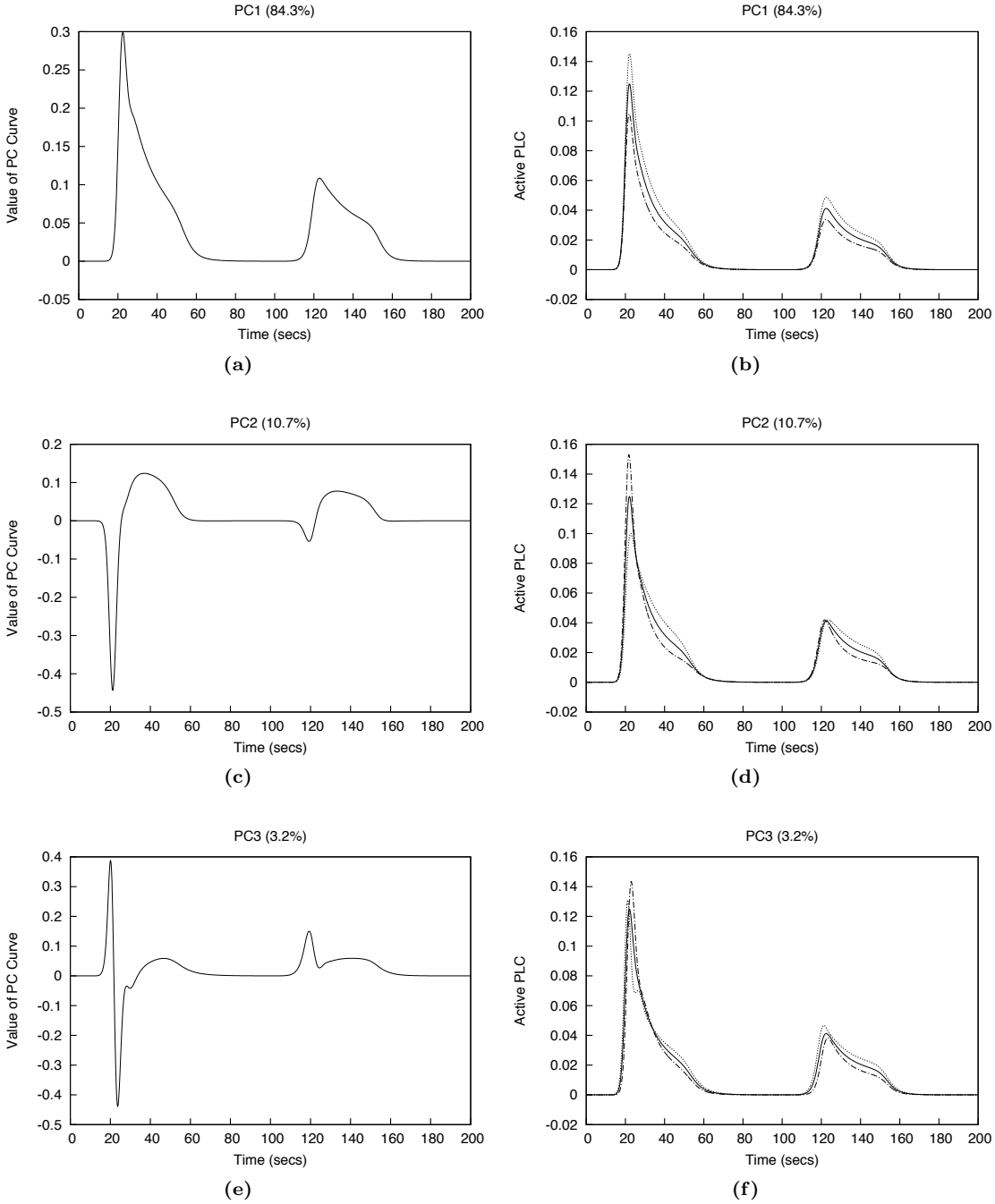


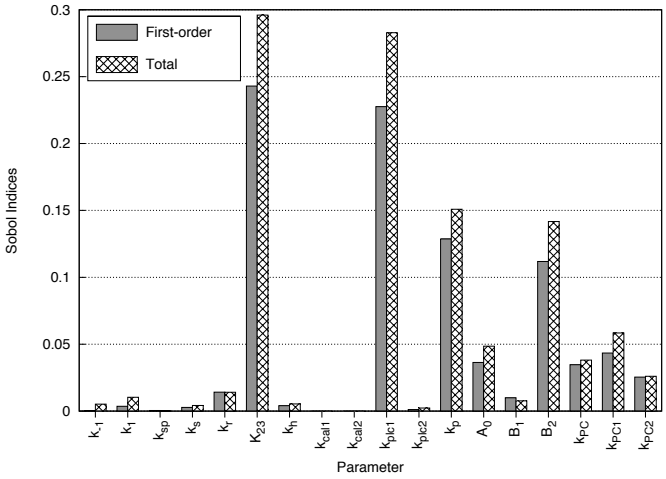
Figure 4.7: The first 3 principal components of the glucagon receptor model output calculated from an expansion of the data using b-splines. Panels a,c, and e show the PC curves. Panels b,d, and f show the mean model output (solid line) plus (dotted line) and minus (dashed line) a multiple of the principal components. The percentages show the amount of the total variation described by each component. Together the first 3 principal components describe 98.2% of the variation in the model output.

PC primarily describes variation in the magnitude of the PLC response to glucagon stimuli. This is the most important mode of variation in the data set. The second component describes the sharpness of the PLC response to both stimuli. Model runs with positive scores on this component have broader peaks of PLC while negative scores are associated with narrow peaks. Principal component three also has an effect on both peaks, but most interestingly shows the existence of a fluctuation in the first peak of PLC.

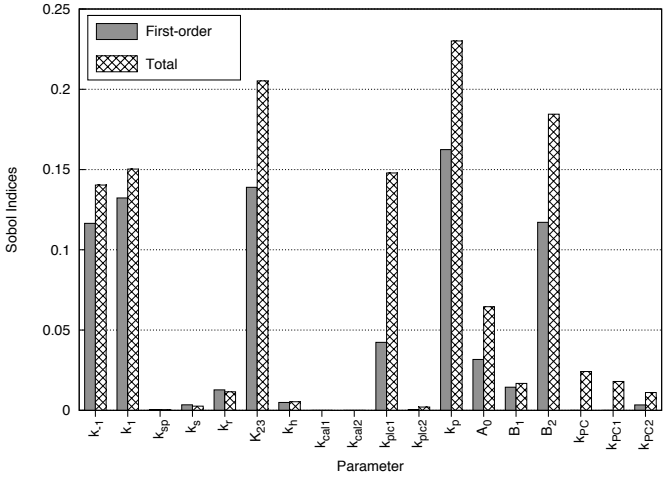
Figure 4.8 shows the first order and total effect indices for the first three PCs of the PLC timecourse. PC1 is most sensitive to K_{23}, the rate of G-protein activation and k_{plc1}, which governs the inactivation of G-proteins by PLC. This suggests that it is the amount of activated G-protein which is most important in driving the magnitude of the PLC response. The importance of k_{plc1} is consistent with experimental evidence that PLC plays an important role in switching off the signal from GPCRs. It achieves this by increasing the activity of GTPases which hydrolyse the GTP bound to active G-proteins resulting in their deactivation (Berstein et al., 1992; Cook et al., 2000). PC1 is also influenced by k_p and B_2 which are involved in the desensitisation of ligand bound receptors. Receptor desensitisation is known to be an important part of GPCR signal transduction, limiting "potentially harmful effects" resulting from prolonged receptor stimulation (Kohout and Lefkowitz, 2003).

The second PC is controlled by the same parameters as PC1 and k_{-1} and k_1, the rates of receptor ligand binding and dissociation. These processes govern the amount of active receptor which in turn produces active G-proteins. This suggests that these parameters play a role in the sharpness of the PLC response.

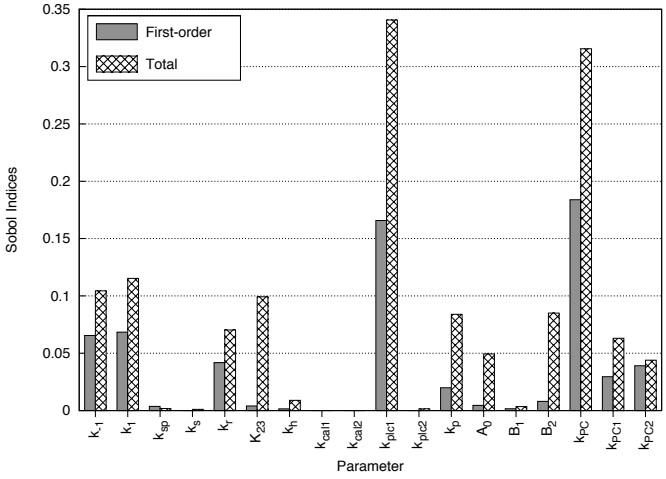
The third PC is largely dependent on k_{plc1} and k_{PC} which determine the deactivation of G-proteins by PLC and the rate of PLC activation by active G-proteins. Both parameters also have significant interaction effects as shown by the difference between their first order and total effect indices. This suggests that the interaction between these parameters may be important in producing the fluctuation in the first PLC peak.



(a) PC1



(b) PC2



(c) PC3

Figure 4.8: The first order and total effect Sobol indices of the glucagon receptor model. The three plots show the indices for the scores of the first three principal components

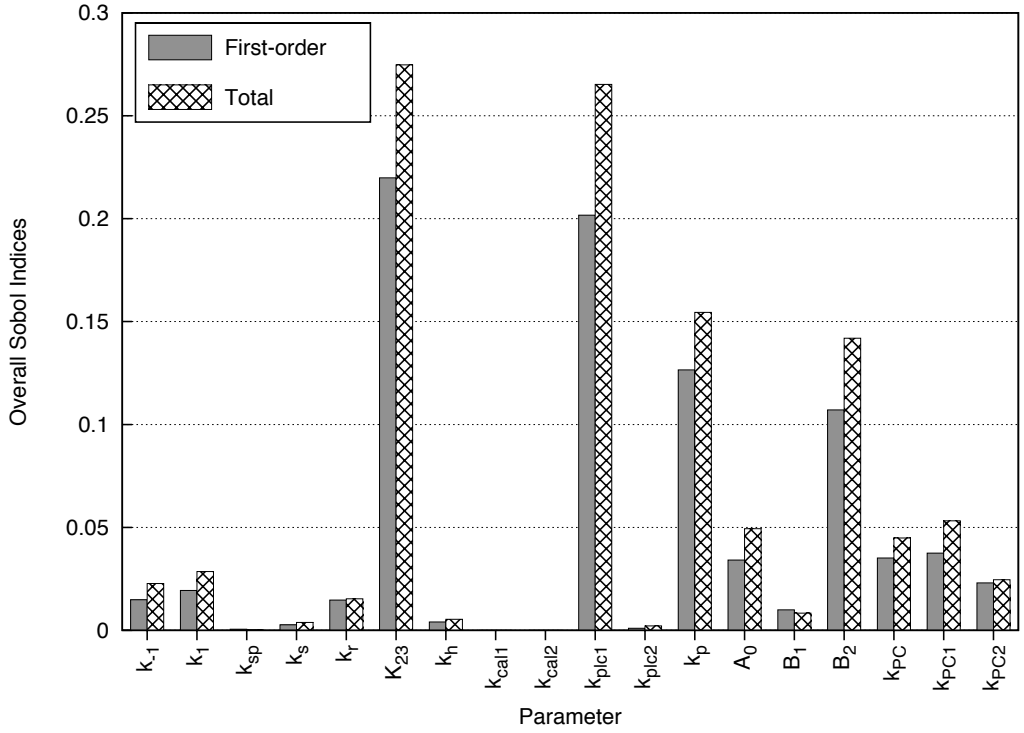


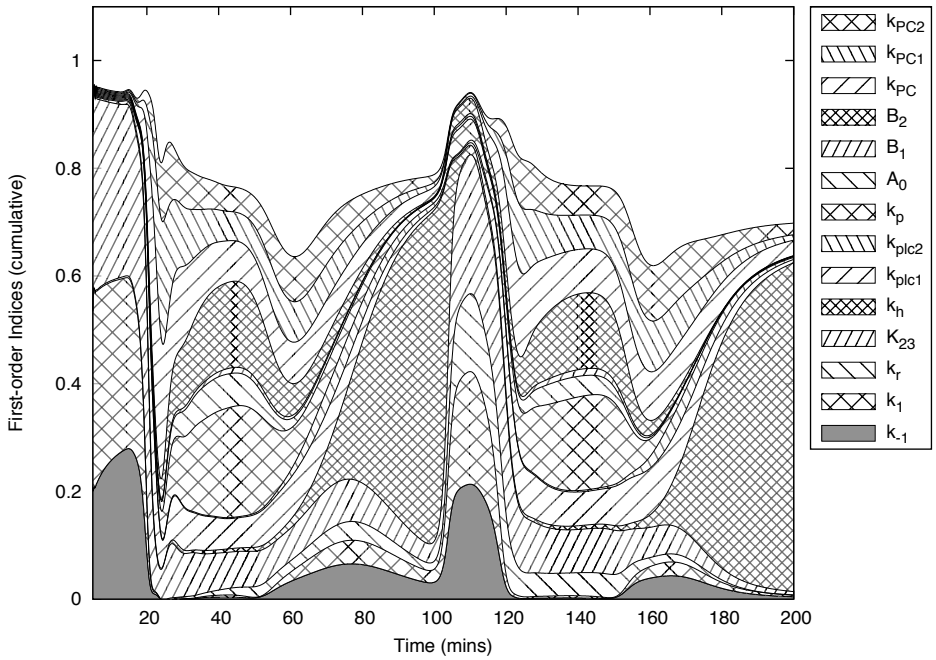
Figure 4.9: The first order and total effect overall Sobol indices of the glucagon receptor model. These describe the effect of parameters on the entire model output.

Figure 4.9 shows the overall sensitivity indices for the glucagon receptor model. The following parameters have minimal effects overall or on any individual component: k_{sp}, k_s, k_r, k_h, k_{cal1}, k_{cal2}, k_{plc2}, A_0, B_1, k_{PC1} and k_{PC2}. This suggests that the sequestration of receptors is unimportant (k_{sp}, k_s, k_r) as is the affect of active G-proteins on the phosphorylation of ligand bound receptors (A_0, B_1). While k_{plc2} is not identified as important, the other parameter involved in the inactivation of G-proteins by PLC (k_{plc1}) is found to be the second most important parameter overall. Perhaps surprisingly, the parameters governing the deactivation of PLC (k_{PC1} and k_{PC2}) have little effect. The lack of sensitivity to k_{cal1} and k_{cal2} is expected as the calcium concentration was set to zero in this analysis. The overall effects of k_{-1}, k_1 and k_{PC} are also small because they only significantly affect the lower order components which describe relatively small amounts of the total variance.

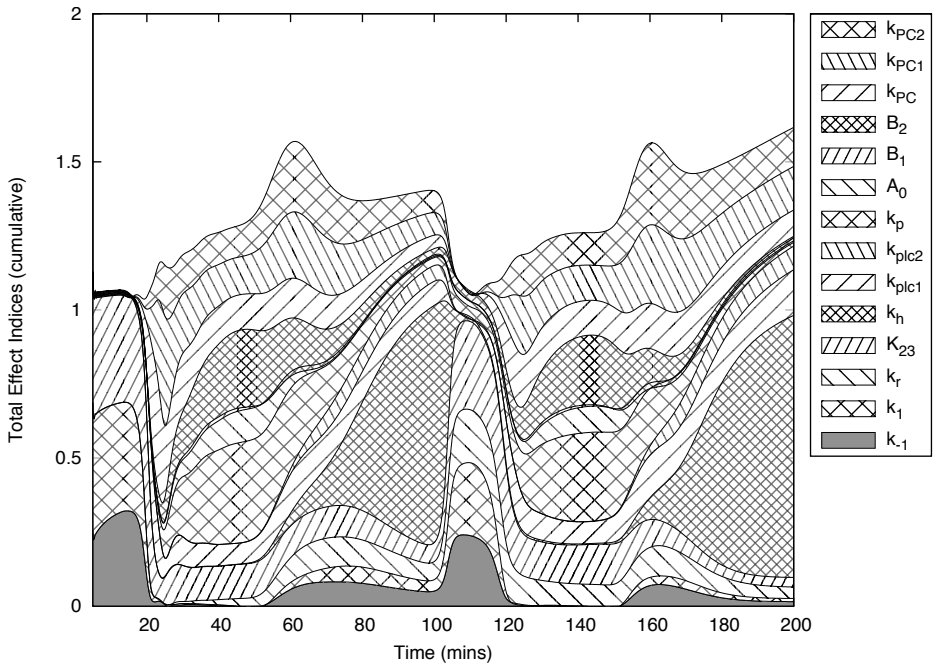
Time Varying Sobol Indices

Figure 4.10 shows the time varying Sobol indices for the glucagon receptor model. Unlike the case of the insulin model where the output was relatively simple it is difficult to relate these results to the different types of variation in the output described by the principal components. It is not obvious from these plots that there is variation in both the magnitude and sharpness of the PLC

response or that it is possible to see a fluctuation in the first response.



(a)



(b)

Figure 4.10: The time varying Sobol indices of the glucagon receptor model. Panel a shows the first order indices, panel b shows the total effects. The indices are displayed as cumulative area plots.

These results also highlight another problem with the use of time-varying sensitivities. Based on figure 4.10 one might assume that k_h was important in determining the behaviour of the model. This view is supported by the time-averaged sensitivities calculated using equation 4.29 and displayed in figure 4.11 which show k_h to be the most important parameter. However, inspection of the model output (figure 4.6) shows that in the periods where k_h is shown to be important (the time between glucagon stimuli) there is minimal variation in the model output. k_h therefore has a minimal effect on the overall variation in the model output as predicted by the use of principal component based sensitivity measures.

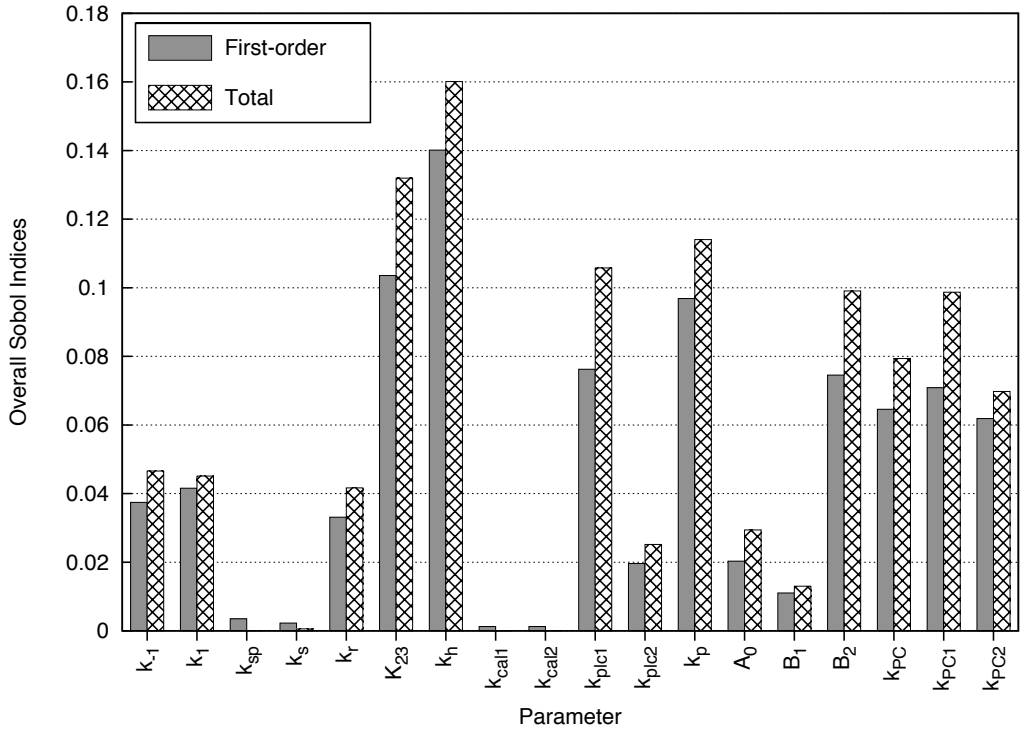


Figure 4.11: The first order and total effect Sobol indices of the glucagon receptor model based on a time average.

4.4 Screening Methods

The preceding section has shown how the variance based method of Sobol can be combined with PCA to investigate the sensitivity of time dependent output of biological models. The main drawback with variance based methods is their computational cost. When a model contains many input factors or takes a long time to evaluate the computational expense may prohibit a complete quantitative sensitivity analysis using such methods. Even in the case of the insulin model, which takes

approximately $t_e = 2$ seconds to evaluate and has $k = 21$ uncertain parameters, the computational time required to generate a full set of first order and total effect Sobol indices is on the order of days on a personal computer (the number of model runs required to generate a single estimate of the S_is and S_{Ti}s using the Sobol method is $N(k + 2)$. The insulin model needed a sample size of $N = 2000$ to ensure convergence of the indices, therefore requiring 46000 model evaluations. At a computational time of 2s per run, this requires approximately 1.06 days).

An efficient alternative to a quantitative analysis is provided by a class of SA methods known as screening designs. The second half of this chapter describes the use of screening methods to investigate the sensitivity of the principal components to the model parameters.

It is often assumed that the number of important factors in a model is small compared with the total number of factors. This is based on the idea that the importance of the factors follows Pareto's Law (Saltelli et al., 2004) which suggests that, for many events, 80% of the effects come from 20% of the causes. Screening designs, defined by Campolongo and colleagues (in Saltelli et al., 2000a, chp. 4) as "any preliminary activity that, independently of the number of experimental runs it uses, aims to discover which of the input factors involved in a model are important, i.e. control most of the output variability", can be used to generate a list of these few important factors which can then be investigated in greater detail.

Typically screening methods are designed to have low computational cost, requiring a small number of model evaluations. The trade off for this economy is that the methods tend to provide qualitative measures of sensitivity, for example ranking the input factors in terms of importance, but give no information about how much more important one factor is than another.

A number of screening designs have been proposed in the literature including one-at-a-time (OAT) methods (in particular the method proposed by Morris (1991)), Cotter's design (Cotter, 1979), iterated fractional factorial design (IFFD) (Andres and Hajas, 1993) and sequential bifurcation (SB) (Bettonvil and Kleijnen, 1997). IFFD and SB are both group-screening techniques (Saltelli et al., 2000a) in which factors are combined into groups prior to the analysis. IFFD produces good results only if a very small number of inputs determine the variability in the model output while SB requires the user to know the signs of the effects (i.e whether a factor has a positive or negative influence on the output) before the analysis is performed. Cotter's design is computationally efficient and requires no prior assumptions about the input/output behaviour of the model but it does have one main drawback. If a factor has effects on the model response which cancel each other out it may not be identified as important by the method (Cacuci et al., 2003). Compared to the other methods, Morris's design (together with the extensions to it proposed by Campolongo et al. (2007)) "has the benefit of a greater applicability" (Saltelli et al., 2004) and has

been shown to be "a very good compromise between accuracy and efficiency" (Campolongo et al., 2007). The method is described in the following section.

4.4.1 Morris's OAT Method

One-at-a-time (OAT) designs evaluate the effect of varying one factor while all others are held constant. The standard approach is to fix each input at some nominal value, often taken from the literature, to define a "control" scenario. Each factor in turn is then varied to two extreme values, usually equidistant either side of the nominal value. The differences between the model outputs at the extreme and control values are then used to rank the inputs.

OAT designs are typically forms of "local" sensitivity analysis. The factors are varied by small amounts around the nominal point and the results of the analysis only identify the model behaviour in the small region of the input space around this point. The results are dependent on the choice of this point and, especially if the model contains strong non-linearities, selection of a different nominal point can produce vastly different outcomes. This problem can be overcome by using the OAT method proposed by Morris (1991).

Morris's OAT method is based on the standard OAT approach described above but removes the dependence on the choice of control point by calculating r local measures for each factor at different nominal points, $\mathbf{X}_1, ..., \mathbf{X}_r$, where $\mathbf{X}_j$ is a k-vector of values $x_{j1}, ..., x_{jk}$ for the k input factors. The nominal points are chosen such that each input is varied over its entire range. The distribution of these individual randomised local measures for a given factor can than be used as an approximation of its global sensitivity. The numerical details of the method are described below.

For a model with k inputs, each of which can take one of p values in the set $\{0, 1/(p-1), 2/(p-1), ..., 1\}$, Morris defines the elementary effect of the i^{th} factor at point $\mathbf{X}$ (note we have dropped the j subscript for convenience) as

$$d_i = \frac{[y(x_1, ..., x_{i-1}, x_i + \Delta, x_{i+1}, ..., x_k) - y(\mathbf{X})]}{\Delta} \tag{4.30}$$

where Δ is a predetermined multiple of $1/(p-1)$ and $\mathbf{X}$ is such that $\mathbf{X} + \Delta$ is still in the set of allowable values for each factor k. The distribution of elementary effects for input i, F_i, can be approximated by generating a random sample of r elementary effects from F_i. r is typically in the region of 10 (Campolongo and Saltelli, 1997; Saltelli et al., 2004). The mean and standard deviation of this sample can be used to assess the importance of the factor. A high mean, μ, indicates an input with an important overall effect on the output. A high standard deviation, σ,

indicates a factor with non-linear effects on the output or one which is involved in interactions with other factors. Plots of μ versus σ can be produced to visualise the results.

The simplest way to generate r samples for k factors requires $2rk$ runs. The model must be run twice for each elementary effect, once at $\mathbf{X}$ and once at $\mathbf{X} + \Delta$. The key to the Morris method is a more efficient design which requires $r(k+1)$ model runs to generate the necessary samples. The method proceeds as follows:

- randomly select a base value $\mathbf{X}^*$ for $\mathbf{X}$, each component being sampled from the subset of possible values $\{0, 1/(p-1), ..., 1-\Delta\}$
- increase one or more of the components of $\mathbf{X}^*$ by Δ such that the resulting vector $\mathbf{X}^{(1)}$ is still in the set of possible values
- generate the second sampling point $\mathbf{X}^{(2)}$ from $\mathbf{X}^*$ with the property that it differs from $\mathbf{X}^{(1)}$ in the randomly selected i^{th} component by $\pm\Delta$.
- select $\mathbf{X}^{(3)}$ such that it differs from $\mathbf{X}^{(2)}$ for only one component $j \neq i$ by $\pm\Delta$

The last step is repeated to produce a succession of $k+1$ input vectors $\mathbf{X}^{(1)}, ..., \mathbf{X}^{(k+1)}$ in which two consecutive vectors differ in only one component and any component i of the base vector has been selected once to be increased by Δ. These $k+1$ vectors form a trajectory in the input space and define a $(k+1) \times k$ matrix $\mathbf{B}^*$ whose rows are the input vectors. If the model is then evaluated for each vector (note that $\mathbf{X}^*$ is not used to evaluate the model), an elementary effect can be calculated for each factor as:

$$d_i(\mathbf{X}^{(l)}) = \frac{[y(\mathbf{X}^{(l+1)}) - y(\mathbf{X}^{(l)})]}{\Delta} \tag{4.31}$$

By generating r such "design" matrices $\mathbf{B}^*$ we can produce a sample of elementary effects of size r for each factor. $\mathbf{B}^*$ can be constructed as follows:

$$\mathbf{B}^* = (\mathbf{J}_{k+1,1}\mathbf{x}^* + (\Delta/2)[(2\mathbf{B} - \mathbf{J}_{k+1,k})\mathbf{D}^* + \mathbf{J}_{k+1,k}])\mathbf{P}^* \tag{4.32}$$

where $\mathbf{B}$ is a $(k+1) \times k$ matrix with elements that are $0s$ and $1s$ such that for every column there are two rows of $\mathbf{B}$ that differ in only one element (a convenient choice is a strictly lower triangular matrix of $1s$), $\mathbf{J}_{k+1,k}$ is a $(k+1) \times k$ matrix of $1s$, $\mathbf{D}^*$ is a k-d diagonal matrix with elements either $+1$ or -1 with equal probability and $\mathbf{P}^*$ is a $k \times k$ random permutation matrix in which

each column contains one element equal to 1 and all others equal to 0 and no two columns have $1s$ in the same position.

As with the Sobol method we can use this approach to investigate the sensitivity of the principal components of the model output. After evaluating the model for each input vector in the r design matrices PCA can be used to find the principal components of the set of $r(k+1)$ model outputs and elementary effects calculated for the scores of each PC.

Extensions of the Morris Method

Two improvements to the Morris method have recently been proposed by Campolongo et al. (2007). The first is the use of an alternative to the mean, μ, as a measure of a factor's importance, the second is an improved strategy for selecting the r design matrices. Both improvements are used in the present study.

If the sample of elementary effects for a given factor contains both positive and negative elements, that is the relationship between the factor and the model output is non-monotonic, they may cancel out producing a low value of μ for an important factor. To overcome this, it has been suggested (Campolongo et al., 2007) that the mean of the absolute values of the elementary effects, denoted μ^* should be used. This modified Morris measure has been shown empirically to be a good proxy for the total effect indices, S_{Ti}, of the variance based measures.

The method proposed by Morris for constructing the r design matrices, $\mathbf{B}^*$, does not ensure that the resulting trajectories will give a good coverage of the input space. The improvement suggested by Campolongo et al. (2007) is first to generate a large number of trajectories $M \approx 500 - 1000$ and then select the r trajectories with the highest "spread". This is achieved by defining the distance between two trajectories as the sum of the geometric distances between all the pairs of points and selecting the set of r trajectories with the greatest total distance between them. This approach improves the coverage of the input space without significantly increasing the computational cost, the model is still only evaluated $r(k+1)$ times.

Scaling of Elementary Effects

In the description of the method given above it was assumed that each input takes values in the range $[0, 1]$. In reality each parameter may have different ranges based on its uncertainty distribution. This can result in different values for Δ for each parameter. In these circumstances it is important to consider the effect of Δ on the elementary effects. As the calculation of elementary effects involves division by the parameter step size, Δ (see equation 4.31) parameters with small values will produce larger effects. This can cause the incorrect classification of the importance of

parameters. This problem can be overcome by applying scaling to the calculation of the elementary effects.

The need to scale the elementary effects was highlighted by Sin and Gernaey (2009). They suggested using "standardized elementary effects" calculated using equation 4.33:

$$d_i(\mathbf{X}^{(l)}) = \frac{[y(\mathbf{X}^{(l+1)}) - y(\mathbf{X}^{(l)})]}{\Delta} \frac{\sigma_{xi}}{\sigma_{yj}} \tag{4.33}$$

where σ_{yj} and σ_{xi} are the standard deviations of outputs y_j (in this case the PC scores) and inputs x_i. The scaling of the elementary effects also removes the dependence on the magnitude of the model output. This allows the effects of a parameter on different outputs to be compared. This is important in the definition of the overall Morris measure (see below). This approach to the scaling of the elementary effects has been implemented in this work.

Overall Morris Measure

As with the PCA based Sobol indices we can define a measure of the overall importance of a parameter on the entire output using the Morris method and the principal components. The overall Morris measure μ^*_{Oi} for parameter i is given by:

$$\mu^*_{Oi} = \sum_{z=1}^{q} \mu_i^{*,z} V_{PC}^z \tag{4.34}$$

While this is not a quantitative measure of the variance described by input i it does provide a weighted measure of the overall effect of parameter i on the model output.

4.4.2 Application of the Morris Method to the Insulin and Glucagon Receptor Models

The Morris method was applied to the insulin and glucagon receptor models using the same parameter ranges and external input functions as used in the application of the Sobol method. After constructing a design matrix, the model was evaluated for each of the $r(k+1)$ input vectors. Principal component analysis was then applied to the set of $r(k+1)$ model outputs and the elementary effects calculated on the principal component scores.

When using the Morris method it is necessary to select appropriate values for p, the number of

levels of each parameter, and r, the number of repetitions. The two are related in that increasing p does not increase the performance of the method unless the number of repetitions is also increased. There is no formal method for selecting r and p, but previous studies have suggested that good results can be obtained using $r = 10$ and $p = 4$ (Campolongo and Saltelli, 1997). Tests on the insulin and glucagon receptor models show that the values $r = 20$, $p = 8$ produce consistent rankings of the parameters.

Insulin Model

Figure 4.12 shows the results of applying the Morris method to the insulin model. Panels a,c and e show the first three principal components. They are qualitatively similar to those calculated during the application of the Sobol method and represent the same types of variation in the model output. They also account for similar proportions of the total output variance. Panels b,d and f show the absolute mean μ^* of the elementary effect against the standard deviation σ for each parameter. The results show that the Morris method identifies the same 10 parameters as important as the Sobol method and that many of the specific features of the quantitative sensitivities are captured.

For the first PC, k_{-3}, k_7 and k_{-7} are found to have a small effect while the remaining parameters of the post receptor signalling pathway are identified as significantly more influential. The effect of non-linearities or interactions is small, as indicated by the relatively low values of σ.

For the second PC, the parameters of the post receptor model are clustered together and found to have more significant non-linear or interaction effects as shown by the higher values for σ. k_{-3} is identified as the most influential factor and also found to have a non-linear or interaction effect.

Panel f shows that k_{-15} is the most influential factor on the third PC as predicted by the Sobol method. The importance of interactions on this PC is also captured by the high values of σ for $k_8, k_{9stim}, k_{-11}, k_{11d}$ and k_{15d}.

The similarity between the ranking of parameters produced by the Sobol and Morris methods is shown in figure 4.13 where the total effect indices of Sobol are plotted against the modified Morris measure μ^*.

The overall effects of the parameters on the model output are shown in figure 4.14. The ranking of the parameters is largely the same as that given by the overall Sobol indices (see table 4.1).

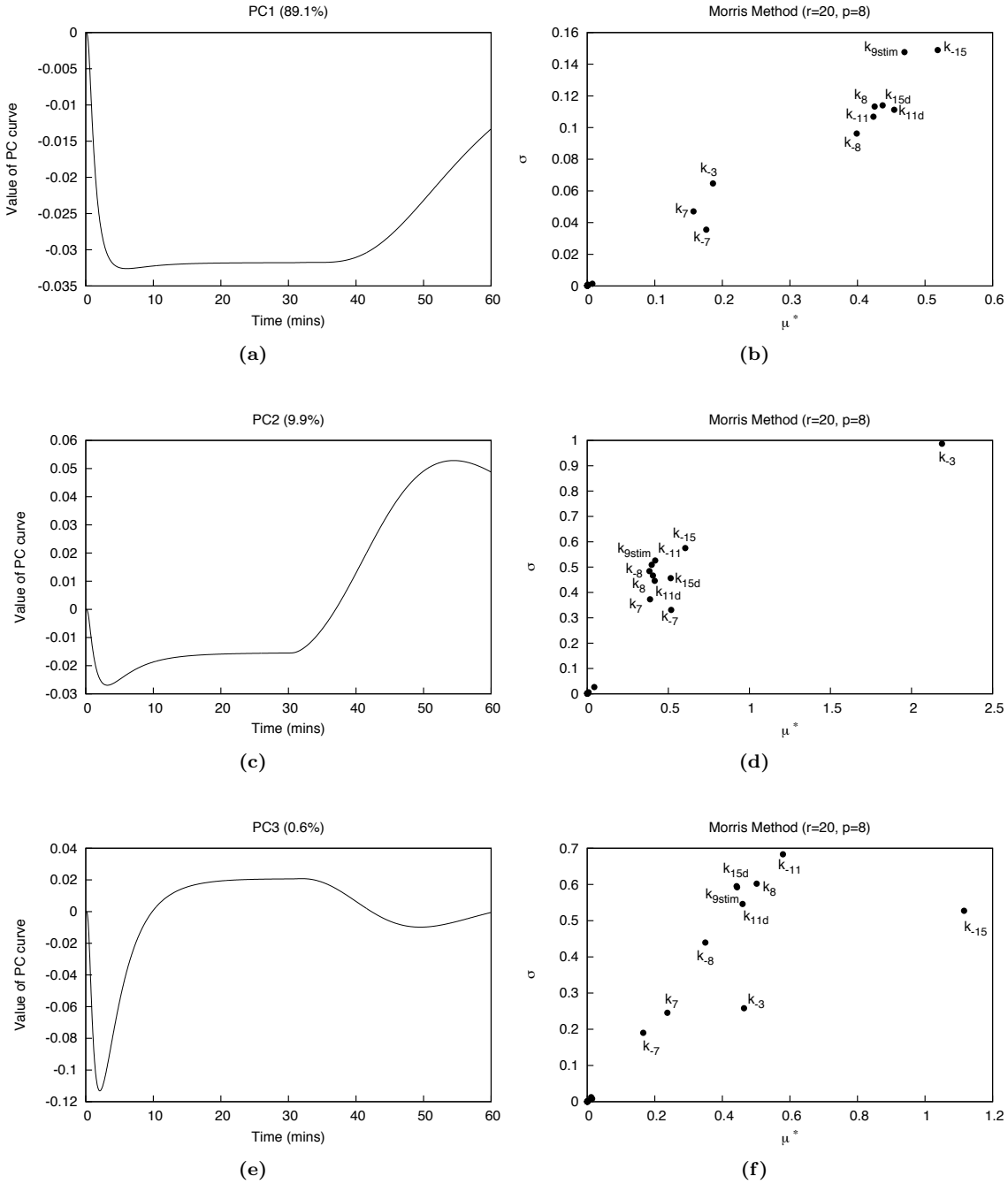
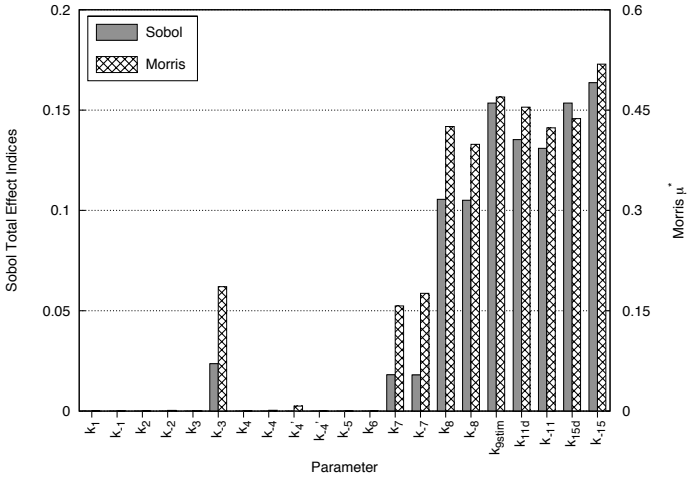
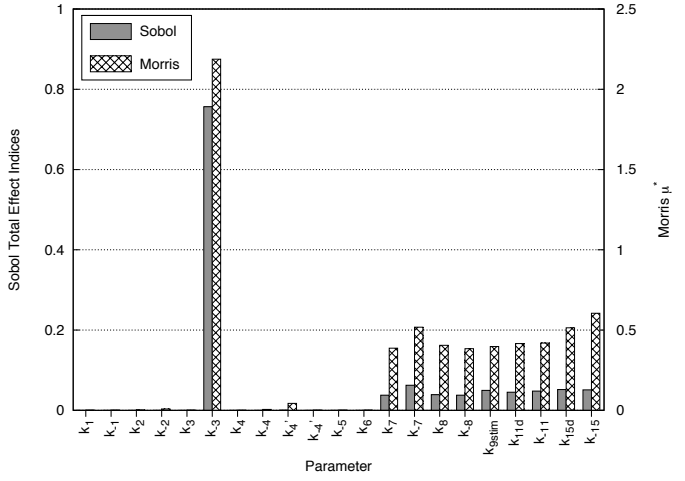


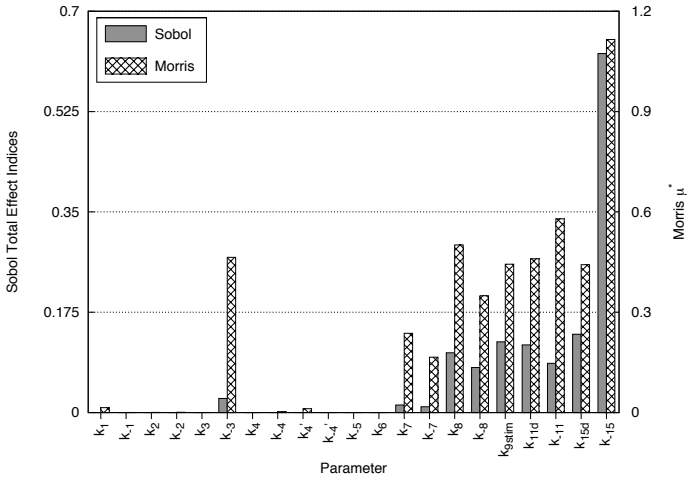
Figure 4.12: The results of applying the Morris method to the insulin model. The left hand figures show the first three principal components extracted from the data set. The variance explained by each PC is shown in brackets. The right hand figures show the mean, μ^*, and standard deviation, σ, of the elementary effects for each input. Number of levels $p = 8$, number of repetitions, $r = 20$.



(a) PC1



(b) PC2



(c) PC3

Figure 4.13: A comparison of the Sobol and Morris methods for the insulin model. The total effect Sobol indices are plotted with the modified Morris measure μ^* for each of the first three PCs.

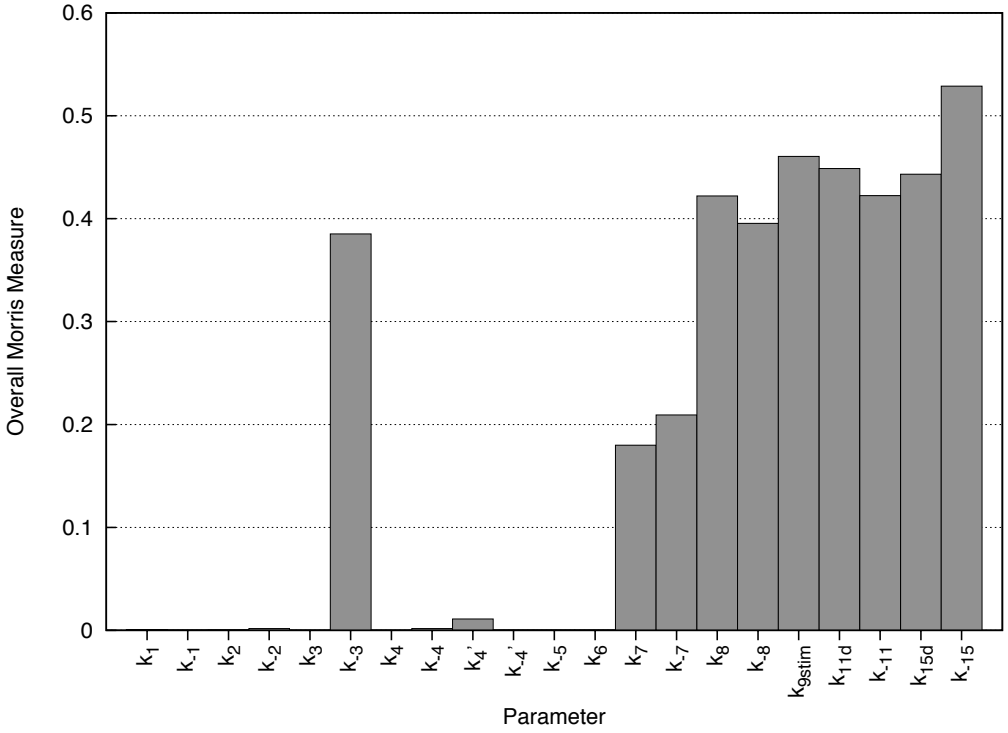


Figure 4.14: The overall Morris measures for the insulin model.

Glucagon Receptor Model

Figure 4.15 shows the principal components and Morris measures for the first three PCs of the glucagon receptor model. As with the insulin model the principal components represent the same type of variation as found via the Sobol method. The mean and standard deviation of the elementary effects show that the same parameters are identified as important in determining the model behaviour. This is shown more clearly in figure 4.16 in which the Sobol and Morris measures are plotted side-by-side. While the ranking is not identical the same subset of parameters is shown to be important by each method.

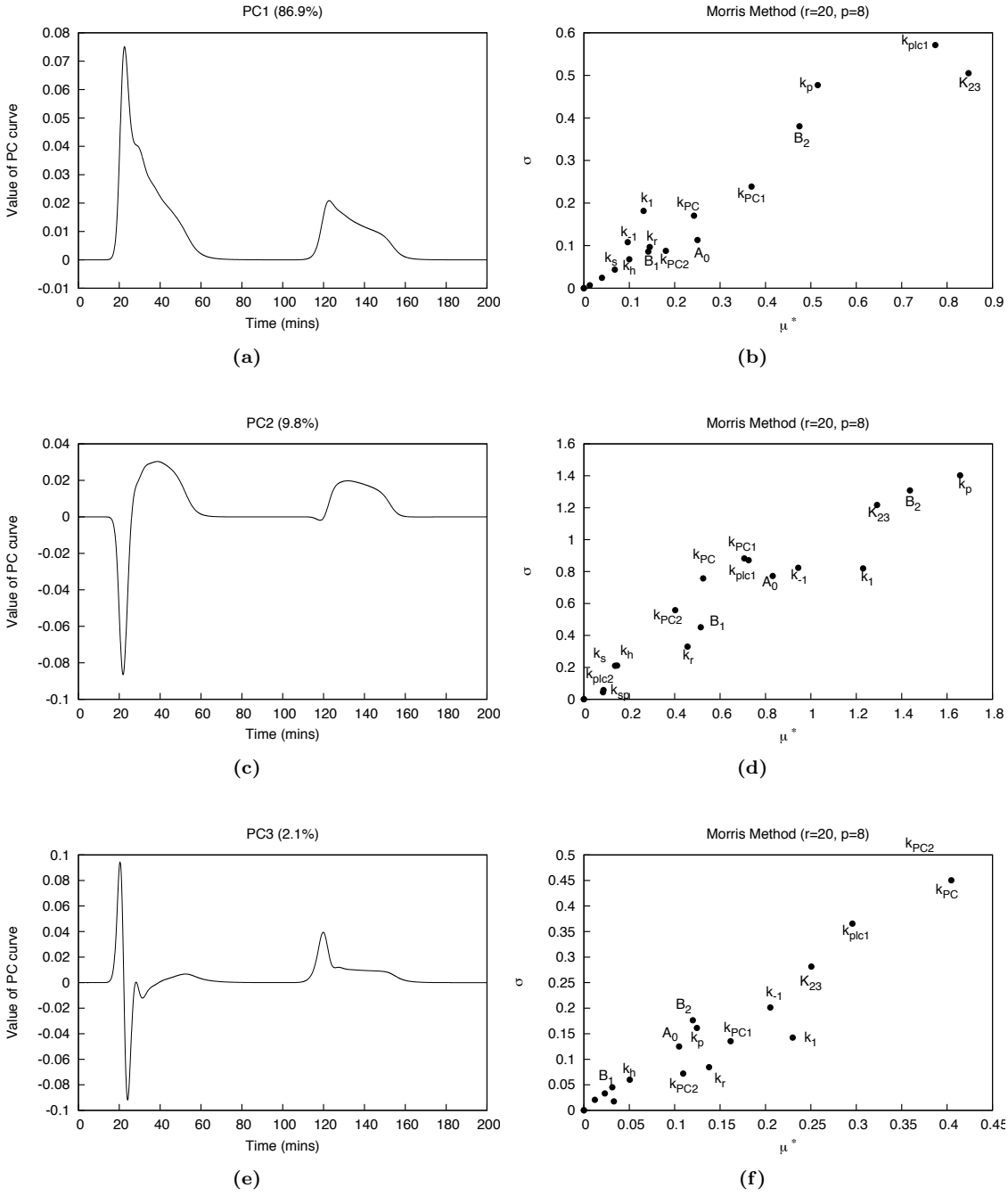
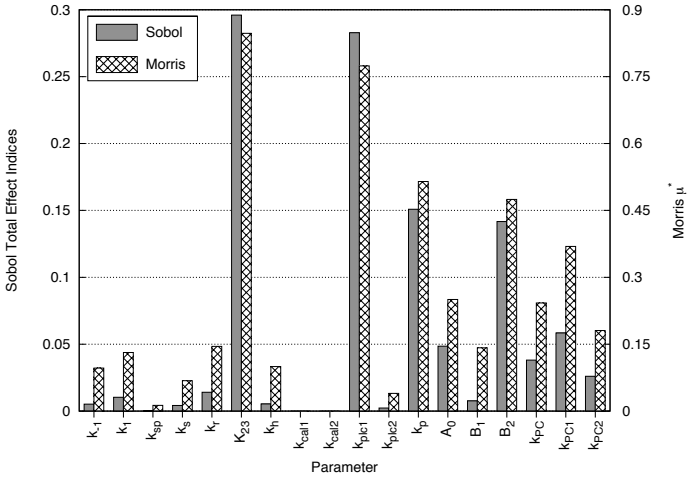
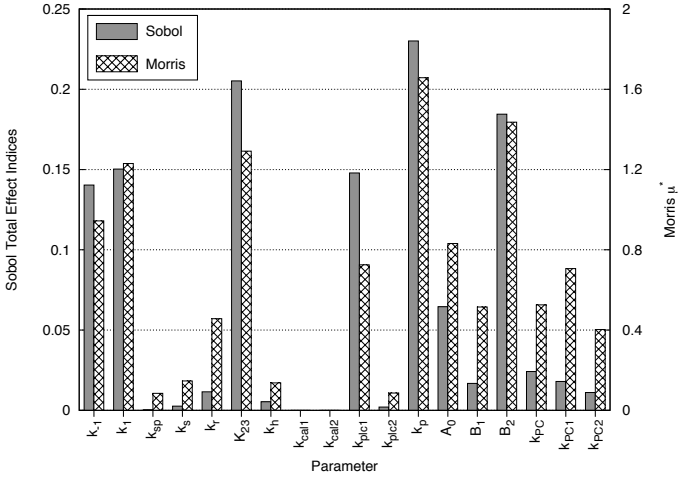


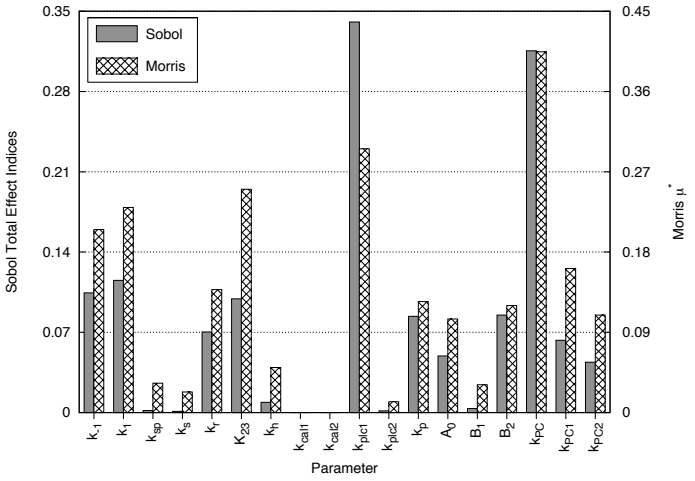
Figure 4.15: The results of applying the Morris method to the glucagon receptor model. The left hand figures show the first three principal components extracted from the data set. The variance explained by each PC is shown in brackets. The right hand figures show the mean, μ^*, and standard deviation, σ, of the elementary effects for each input. Number of levels $p = 8$, number of repetitions, $r = 20$.



(a) PC1



(b) PC2



(c) PC3

Figure 4.16: A comparison of the Sobol and Morris methods for the glucagon receptor model. The total effect Sobol indices are plotted with the modified Morris measure μ^* for each of the first three PCs.

The overall effects (figure 4.17) also show the same qualitative information as the Sobol overall indices (see figure 4.9).

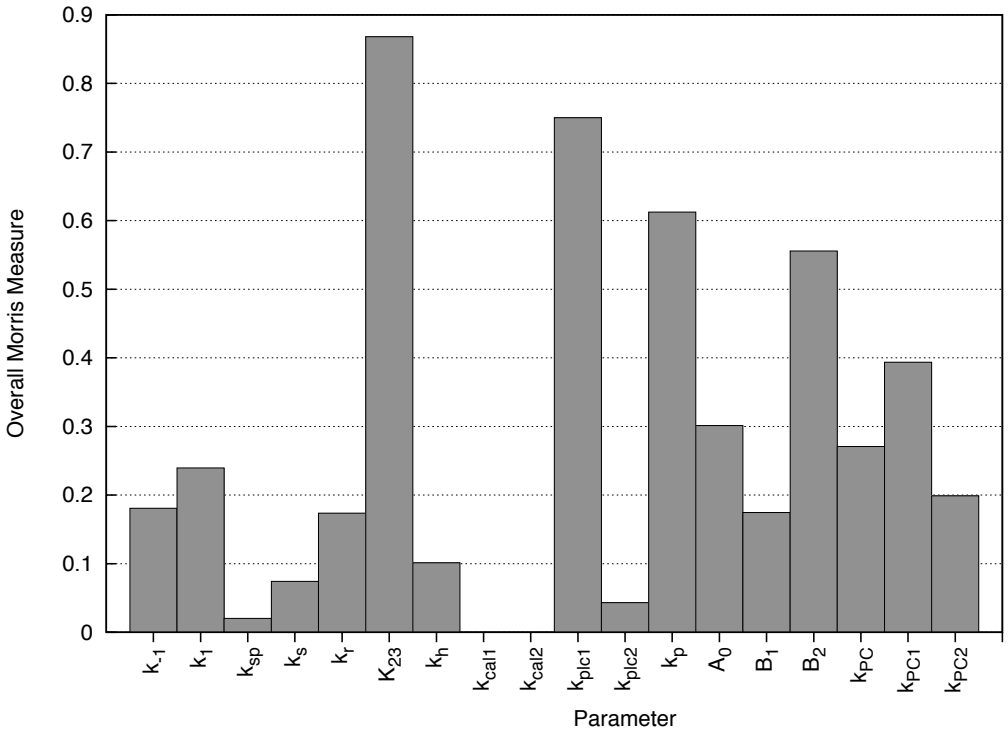


Figure 4.17: The overall Morris measures for the glucagon receptor model.

4.4.3 Computational Times

This section has shown that the Morris method produces sensitivity information which is consistent with the results of the variance based method of Sobol. Importantly the computational time required to calculate these results is of the order of tens of minutes, significantly less than the Sobol method. The main computational effort of both methods is the evaluation of the model. The two methods require $r(k+1)$ and $N(k+2)$ model runs respectively. Typical values for r are of the order of 10 (Campolongo and Saltelli, 1997; Saltelli et al., 2000a) while N is of the order of a thousand. For a given model, the Morris method therefore requires fewer model runs and hence has a lower computational cost. As an example, the computational time for the insulin model ($k = 21$) using the Morris method ($r = 20$) is approximately 15 minutes while for the Sobol method ($N = 2000$) it is 1.06 days. For the glucagon receptor model ($k = 18$) the Morris method ($r = 20$) takes approximately 9.5 minutes to evaluate the model compared with 16.7 hours for the Sobol method ($N = 2000$).

The reduced computational cost of the Morris method means it is practical to run the analysis numerous times, for example to study the effects of different external inputs on the sensitivity of the model.

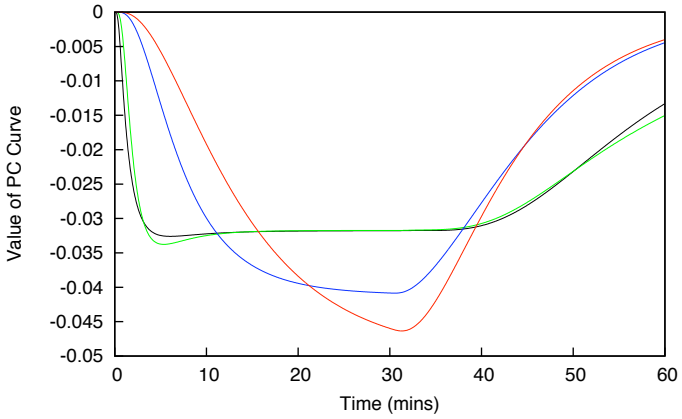
4.4.4 Investigating the Effect of the Input Function

The behaviour of the insulin model (and any model in general) will depend on the external input, in this case the insulin concentration. It is also possible that the sensitivities are dependent on the external model input as found by Liu et al. (2005) when using a local sensitivity analysis to study a model of epidermal growth factor mediated signalling. To investigate this potential dependence the Morris method was applied to the insulin model for a range of insulin concentrations. Figures 4.18 and 4.19 show how the principal components and their sensitivities (measured by μ^*) depend on the concentration. The principal components are qualitatively similar and describe the same types of variation for each insulin concentration. The distribution of the total variance is also similar.

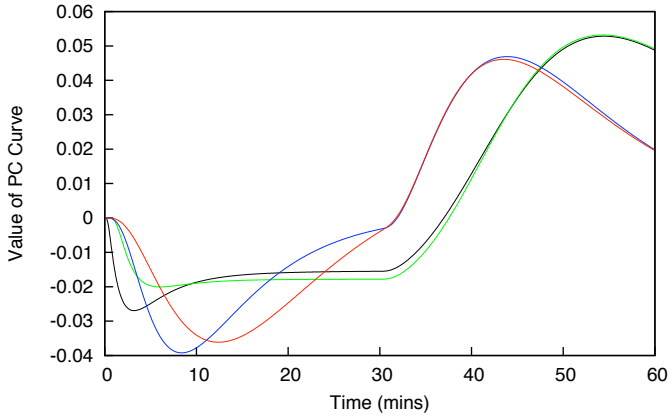
The most obvious difference in the sensitivities is the increased importance of k_1, the association constant for the first insulin molecule, as the insulin concentration is changed. It is realistic that at lower insulin concentrations, when there is insufficient ligand to saturate the receptor the affinity of the receptor for insulin would have a greater effect on the model output. However, it should be noted that the relationship between the importance of k_1 and the insulin concentration appears to be non-monotonic.

These results show the importance of considering the effect of different scenarios or input functions on the sensitivity of the model. Depending on the external inputs to the model, different parameters are identified as important.

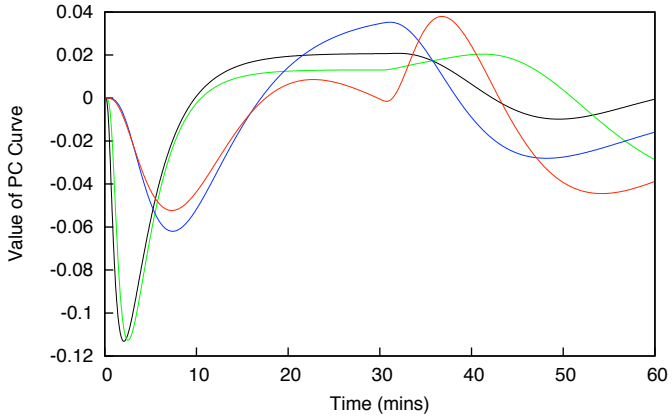
In the case of the composite model described in chapter 3 the external input to a given component model is dependant on the behaviour of the other sub-models. It is therefore necessary to study the complete model to understand the sensitivity of the system to uncertainties in the component model parameters. The next chapter discusses approaches to this task.



(a) PC1

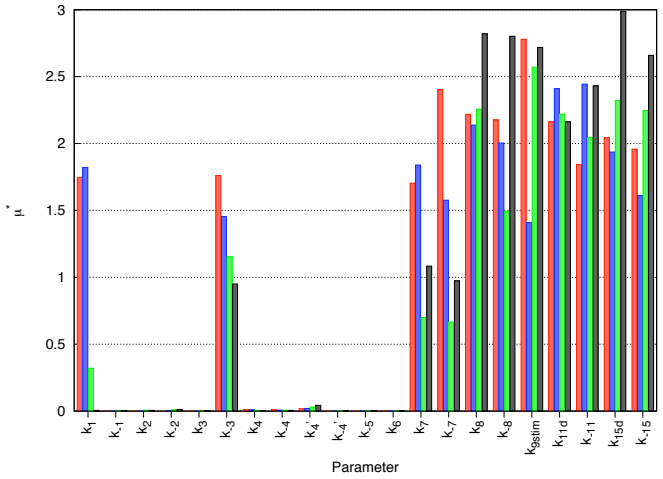


(b) PC2

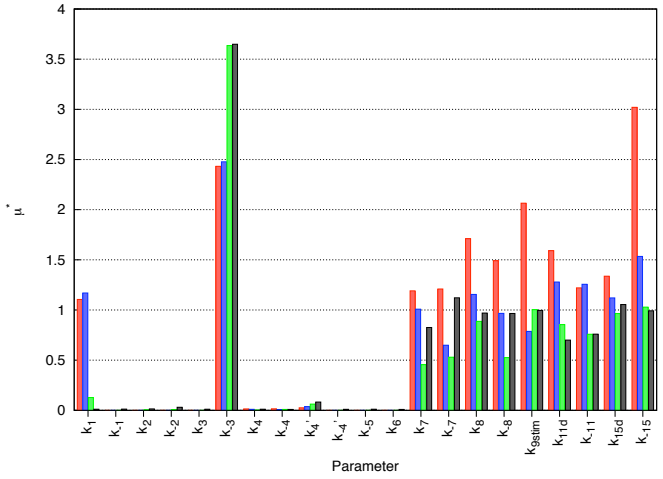


(c) PC3

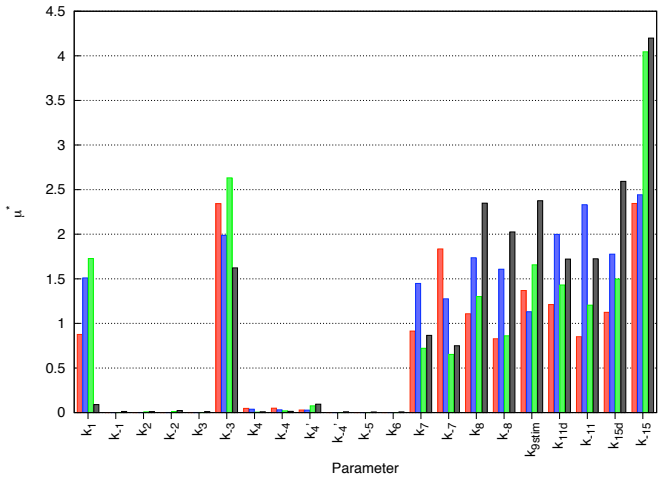
Figure 4.18: The effect of the external insulin concentration on the first three principal components of the insulin model. (1×10^{-12} (red), 1×10^{-10} (blue), 1×10^{-8} (green), 1×10^{-6} (black)).



(a) PC1



(b) PC2



(c) PC3

Figure 4.19: The effect of the external insulin concentration on μ^* for the first three PCs. (1×10^{-12} (red), 1×10^{-10} (blue), 1×10^{-8} (green), 1×10^{-6} (black)).

4.5 Discussion and Conclusions

In biological modelling we are often interested in the dynamic output of a model. However, the current approaches to the sensitivity analysis of dynamic model outputs have drawbacks. In particular the use of time-varying sensitivities makes it difficult to draw conclusions about the effects of parameters on specific features of the model output. This chapter has discussed an alternative approach to performing this analysis. The method, based on a suggestion by Campbell et al. (2006), uses a principal component analysis of the functional model output to extract the key modes of variation from the data. Sensitivity analysis is then applied to the principal component scores to investigate how these modes depend on the model parameters.

In section 4.3 this approach was developed using the variance based method of Sobol to perform the sensitivity analysis. The Sobol method is a global SA technique which quantifies the fraction of the total variance in the model output accounted for by each parameter, both on its own and through its interactions with other parameters. The PC based approach was applied to both the insulin and glucagon receptor sub-models of the multi-scale glucose homeostasis model described in chapter 3.

The results were compared to the time-varying sensitivities also calculated using the Sobol method. The results demonstrate that the PC based measures provide additional information on important behaviours of the model which would not be inferred from the time-varying indices. This is a key benefit of the use of principal components; the features are extracted directly from the data and are based on the important variation in that data rather than predetermined ideas of what is important.

The use of principal component analysis also allows the definition of an overall sensitivity, the importance of a parameter on the entire model output. This overall sensitivity weights the modes of variation described by the PCs by the amount of variance they account for. This avoids the problem encountered when using time-varying sensitivities of incorrectly identifying a parameter as important when it describes little of the variance in the overall output.

The main drawback of the Sobol method is its computational cost particularly when a model contains a larger number of parameters. In the second half of this chapter the use of a more efficient sensitivity analysis technique has been presented. The Morris method is a screening design which provides a measure of the sensitivity of parameters at a reduced computational cost. The method is based on a series of local measures or elementary effects and provides a qualitative measure of the overall importance of a parameter and its non-linear or interaction effects.

The Morris method was applied to the insulin and glucagon receptor models using the principal

component scores as the model "output". The method identified both the same important modes of variation and the same important parameters as the more costly Sobol method and provides a practical approach to the analysis of models containing large numbers of parameters.

The efficiency of the Morris method means it can be used to perform repeat analysis, for example to study the effects of the external inputs to a model on the sensitivities. Analysis of the insulin model showed that the sensitivities are dependent on the insulin concentration. In a composite model this input will be determined by the behaviour of the other component models highlighting the importance of studying the complete system. This will be discussed in more detail in chapter 5.

A similar approach to the sensitivity analysis of dynamic model output has recently been utilised by Lurette et al. (2009) to study an epidemiological model describing the infection dynamics of salmonella in pigs. The methodology was presented by Lamboni et al. (2008) in a technical report of the French National Institute for Agricultural Research where it was applied to an agronomic model of wheat production. The method used a factorial design for the model parameters and an analysis of variance (ANOVA) to calculate the contributions of individual parameters and two-factor interactions to the variance in the principal component scores. The sensitivity analysis methods presented here have the advantage of capturing higher order interactions.

There are limitations to the approach described in this chapter. The approach can be considered as exploratory. Its major benefit is that it allows us to identify the most important types of variation in the model output and investigate how these types of variation are sensitive to the model parameters without any prior assumptions. If however we are interested in a specific type of behaviour of the model there is no guarantee that it will be well captured by the principal components because it may not be important in terms of the main variation in the data. Alternatively it may be combined with other types of variation into a single principal component. This is particularly the case in the higher order effects where multiple physical effects may be described by a single PC. A better approach to this type of analysis is to implement a computational algorithm to extract a scalar measure of the feature of interest directly from the data and apply sensitivity analysis to this value. This approach has previously been used in the analysis of biological systems (Ihekwaba et al., 2004; Hetherington et al., 2006b). In such cases it may be useful to complement the specific study with a PCA based analysis. Because the main computational cost of any sensitivity analysis is in the evaluation of the model this will not add significantly to the time required for the analysis.

A second potential limitation is the ability of principal component analysis to deal with a set of model outputs which display very different behaviours. This issue was highlighted by Jones

and Rice (1992) in their use of PCA to investigate the variation in clusters of "similar" curves. It was suggested that individual curves which are "wildly different" from the general trend would significantly influence the output of PCA and should be removed by inspection before performing the analysis. This problem has not been observed in the set of models analysed in this thesis and it is not apparent where such a situation might arise in the output of a deterministic computational model. However it is a potential issue which should be considered in future applications of the approach.

Chapter 5

Sensitivity Analysis of Composite Biological Models

This chapter discusses approaches to the sensitivity analysis of composite models consisting of multiple component models at one or more scales. Two complimentary approaches are presented. The first uses a group sensitivity analysis to investigate the importance of the various component models. This is followed by a discussion and demonstration of the concepts of intra and inter sensitivities which can be used to study the propagation of individual parameter uncertainties throughout a composite model.

5.1 Introduction

Biological systems, such as the signalling pathways analysed in chapter 4, do not operate in isolation but form part of larger systems involving processes at a variety of temporal, spatial and biological scales. The output of a given sub-system can affect, and be affected by, the function of other sub-systems and the behaviour of the complete system is dependent on these interactions.

Mathematical and computational models are a powerful tool for studying multi-component or multi-scale systems. They allow us to combine information from different levels into a complete description of the system. One way to construct such models is to adopt a modular approach, combining "component" models of the various sub-processes to form a "composite" model describing the entire system in which component models are connected via their output variables which act as inputs to other sub-models.

Composite models may contain large numbers of uncertain parameters and have complex struc-

tures in which the effects of uncertainties and perturbations will not be obvious. Uncertainty in a component model parameter will not only influence the component model output but may be propagated through the system, affecting the output at other levels. Sensitivity analysis provides a way to systematically investigate the effects of such uncertainties.

Most applications of sensitivity analysis in biological modelling have focussed on a single component or scale, typically intracellular signalling pathways. This chapter presents approaches to the analysis of composite models which include multiple component models at one or more scales. The next section describes the use of group sensitivity analysis to investigate the importance of component models in controlling the system level behaviour. The second half of the chapter introduces the concepts of intra (within sub-model) and inter (between sub-model) sensitivities and shows how these can be used to investigate the effect of individual parameters on the function of a composite model. The work presented in this chapter makes use of the principal component based approach for the sensitivity analysis of time dependent model output presented in chapter 4.

5.2 Group Sensitivity Analysis

Group sensitivity analysis refers to the use of SA techniques to look at the sensitivity of a model output to groups of parameters. The ability to work with groups of parameters is a desirable feature of sensitivity analysis techniques because it reduces the computational cost of the analysis. If a model contains a large number of parameters it may not be practical to perform a complete analysis. By considering groups of parameters the number of model evaluations required can be reduced; the number of parameters k is replaced with the number of groups $G < k$ in formulae for the number of model runs needed to calculate the sensitivity measures. This reduced computational demand means it is practical to perform the analysis. However the increased efficiency comes at the cost of information about the importance of the individual parameters belonging to a group.

This section shows how a group SA approach can be utilised to investigate the sensitivity of composite models by treating the parameters of each component model as a group. In addition to increasing the computational efficiency of the analysis (which may be important for composite models containing large numbers of parameters) the use of group SA allows us to study the sensitivity of the composite model to uncertainty in the output of each component model rather than individual parameters. This information can be used to identify the component models which are most important in determining the composite model behaviour. The rest of this section shows how the Morris method can be used to perform group sensitivity analysis and demonstrates its application on a biological model.

5.2.1 The Morris Method on Groups

The ability to work with groups is an important feature of the variance based methods, including the method of Sobol. It has also been shown (Campolongo et al., 2007) that the Morris method can be used to look at groups of parameters. Due to the computational efficiency of the Morris method and its ability to provide results which are consistent with the more expensive variance based techniques (see chapter 4) this method was chosen to investigate the potential of group SA in the analysis of composite biological models.

The Morris method, which was introduced in chapter 4, approximates a global sensitivity measure by calculating r local measures, referred to as elementary effects, for each uncertain parameter. For a model with k parameters, each of which can take one of p values in the set $\{0, 1/(p-1), 2/(p-1), ..., 1\}$, the elementary effect of the i^{th} parameter at point $\mathbf{X} = (x_1, ..., x_k)$ is:

$$d_i = \frac{[y(x_1, ..., x_{i-1}, x_i + \Delta, x_{i+1}, ..., x_k) - y(\mathbf{X})]}{\Delta} \tag{5.1}$$

where Δ is a predetermined multiple of $1/(p-1)$ and $\mathbf{X}$ is such that $\mathbf{X} + \Delta$ is still in the set of allowable values for each parameter. The r elementary effects are calculated at different points in the input space which are chosen so that each parameter is varied over its entire range. The mean and standard deviation of the elementary effects give a measure of the global importance of the parameter. A high mean implies a parameter is important and a high standard deviation means its effect is non-linear or a result of interactions with other parameters.

To extend the Morris method to groups of parameters all the parameters belonging to a group must be moved simultaneously before we re-evaluate the model and calculate the elementary effect. Consider the case of a group of two parameters, $\mathbf{u} = (x_1, x_2)$. The elementary effect at point $\mathbf{X}$ is given by:

$$|d_u(\mathbf{X})| = \frac{|y(\hat{\mathbf{X}}) - y(\mathbf{X})|}{\Delta} \tag{5.2}$$

where $\hat{\mathbf{X}}$ is a point in the input space in which $\hat{x}_1, \hat{x}_2$ have been either increased or decreased by Δ with respect to x_1, x_2. As individual parameters can be increased or decreased by Δ it is necessary to use absolute elementary effects, one of the improvements to the original method proposed in Campolongo et al. (2007).

The key to the Morris method is the algorithm for generating the input points which is designed to minimise the number of model runs required to obtain a sample of elementary effects for each parameter. Details of the procedure are given in chapter 4. To apply the method to groups of inputs it is necessary to modify the original algorithm to allow groups of parameters to be changed while still minimising the number of model evaluations required. Campolongo et al. (2007) did not provide details of their algorithm so a suitable approach had to be developed here.

Computational Algorithm

To generate the inputs for the group Morris method it was decided to use a two stage process. First the order in which the groups of parameters will be perturbed is determined. This is achieved by generating a G x G random permutation matrix, $\mathbf{G}^*$ (where G is the number of groups). This gives us a randomised order in which to change the groups of parameters.

The original algorithm (see equation 4.32) is then used to generate an input matrix $\mathbf{B}^*_\mathbf{j}$ of size k_j for each group $j = 1, ..., G$ in which each element will have been increased or decreased by Δ (k_j is the number of parameters in group j). The first and last rows of these matrices gives us an initial and modified point for each group. These values are then combined, in an order determined by the permutation matrix $\mathbf{G}^*$, to give us one input trajectory. This procedure is repeated r times where r is the number of effects we wish to calculate for each group. The model is then evaluated at each point and the absolute elementary effects are calculated.

The algorithm is illustrated by a simple example. Consider a model with 7 parameters divided into $G = 3$ groups with $k_1 = 3, k_2 = 2$ and $k_3 = 2$. We assume each parameter varies in the range $[0, 1]$ and can take $p = 3$ values, $0, 1/2, 1$. First we generate a G x G permutation matrix:

$$\mathbf{G}^* = \begin{bmatrix} 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

This says we will move group 2 then group 1 and finally group 3. We then generate the three Morris matrices $\mathbf{B}^*_\mathbf{j}$, $j = 1, ..., 3$:

$$\mathbf{B_1^*} = \begin{bmatrix} 0 & 1/2 & 0 \\ 0 & 1/2 & 1/2 \\ 1/2 & 1/2 & 1/2 \\ 1/2 & 1 & 1/2 \end{bmatrix}, \mathbf{B_2^*} = \begin{bmatrix} 1 & 1/2 \\ 1 & 1 \\ 1/2 & 1 \end{bmatrix}, \mathbf{B_3^*} = \begin{bmatrix} 0 & 1 \\ 1/2 & 1 \\ 1/2 & 1/2 \end{bmatrix}$$

The first and last rows of these matrices represent the initial and perturbed parameter points for each group. Finally we combine these rows, based on the order in $\mathbf{G}^*$, to produce one group input trajectory:

$$\mathbf{B}^* = \begin{bmatrix} 0 & 1/2 & 0 & 1 & 1/2 & 0 & 1 \\ 0 & 1/2 & 0 & 1/2 & 1 & 0 & 1 \\ 1/2 & 1 & 1/2 & 1/2 & 1 & 0 & 1 \\ 1/2 & 1 & 1/2 & 1/2 & 1 & 1/2 & 1/2 \end{bmatrix}$$

The first row of $\mathbf{B}^*$ is the initial parameter point. In the second row the $k_2 = 2$ parameters of group 2 have been changed to their perturbed values. In the third row the $k_1 = 3$ parameters of group 1 have also been changed and in the final row the k_2 parameters of group 3 are changed.

Test Case

To test the implementation of the Morris method on groups it was applied to the g-function (Sobol, 1993). This function is commonly used as a benchmark for sensitivity analysis methods because it is possible to calculate analytical values for the variance based sensitivity indices.

$$g = \prod_{i=1}^{k} g_i(X_i), \text{ where } g_i(X_i) = \frac{|4X_i - 2| + a_i}{1 + a_i} \tag{5.3}$$

where $0 \leq X_i \leq 1$, $i = 1, ..., k$ are the uncertain inputs, uniformly distributed in the range [0,1], and the $a_i \geq 0$ are fixed parameters which determine the relative importance of the X_i. The smaller the value of a_i the more important X_i is in determining the value of g.

For the test a g-function with 9 inputs was used. Three different scenarios were considered. In each case the parameters were divided into three groups u, v, w. The groupings are designed to test the ability of the approach to handle different mixtures of important and unimportant parameters. Details of the parameter groups, the associated a_is and the results of applying the method are given below together with the analytical Sobol indices.

Case 1

Parameters a_i

$a_1 = 0.02, a_2 = 0.03, a_3 = 0.05, a_4 = 11, a_5 = 12.5, a_6 = 13, a_7 = 34, a_8 = 35, a_9 = 37$

Groups

$u = [X_1, X_2, X_3]$, $v = [X_4, X_5, X_6]$, $w = [X_7, X_8, X_9]$

Morris Group μ^*

$\mu^*(u) = 7.58564$, $\mu^*(v) = 1.23745$, $\mu^*(w) = 0.17666$

Analytical Sobol Indices S_T

$S_T(u) = 0.995, S_T(v) = 0.010, S_T(w) = 0.001$

Case 2

Parameters a_i

$a_1 = 0.02, a_2 = 0.03, a_3 = 0.04, a_4 = 0.05, a_5 = 0.06, a_6 = 0.07, a_7 = 34, a_8 = 35, a_9 = 37$

Groups

$u = [X_1, X_3, X_5]$, $v = [X_2, X_4, X_6]$, $w = [X_7, X_8, X_9]$

Morris Group μ^*

$\mu^*(u) = 10.62385$, $\mu^*(v) = 10.42485$, $\mu^*(w) = 0.24845$

Analytical Sobol Indices S_T

$S_T(u) = 0.694, S_T(v) = 0.686, S_T(w) = 0.001$

Case 3

Parameters a_i

$a_1 = 0.02, a_2 = 0.03, a_3 = 0.05, a_4 = 11, a_5 = 12.5, a_6 = 13, a_7 = 34, a_8 = 35, a_9 = 37$

Groups

$u = [X_1, X_4, X_8]$, $v = [X_3, X_5, X_9]$, $w = [X_2, X_6, X_7]$

Morris Group μ^*

$\mu^*(u) = 4.4971$, $\mu^*(v) = 3.3537$, $\mu^*(w) = 3.7821$

Analytical Sobol Indices S_T

$S_T(u) = 0.436, S_T(v) = 0.393, S_T(w) = 0.429$

The results of the test case show that the method produces rankings which are consistent with the Sobol method. The results are also in agreement with the results obtained by Campolongo et al. (2007) who used the same test case to demonstrate the potential of the Morris method for performing group sensitivity analysis. These tests indicate that the algorithm outlined above and its implementation are correct. The next section discusses the application of the method to a biological model.

5.2.2 Application of the Group Morris Method to The Insulin Model

To investigate the utility of the group approach for studying composite biological models it was applied to the insulin component model. The insulin model is taken from Sedaghat et al. (2002) and modified to describe GSK3 inactivation as described in chapter 3. The model makes use of two previously published models of receptor binding (Wanant and Quon, 2000) and receptor recycling (Quon and Campfield, 1991) together with a model of the post-receptor signal propagation. A schematic representation of the model is shown in figure 3.4. For the purposes of demonstrating the group approach this can be viewed as a composite model consisting of three component models.

To perform the analysis the parameters were divided into three groups, u, v and w, associated with the three sub-models:

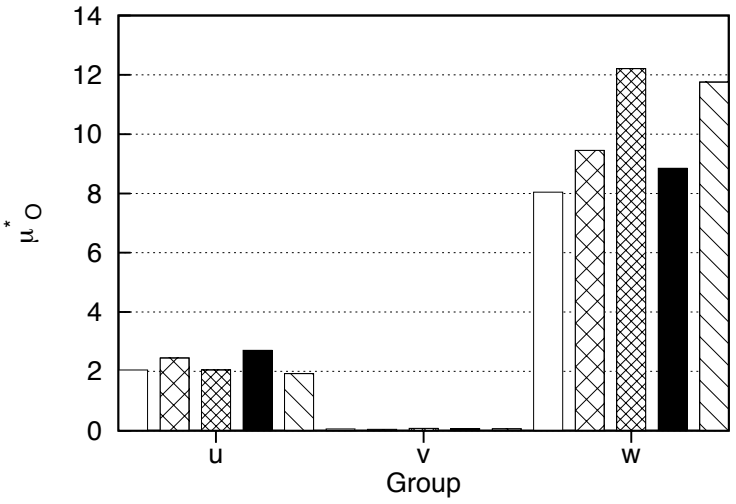
- u = Parameters in the ligand-receptor binding sub-system
- v = Parameters in the receptor recycling subsystem
- w = Parameters in the post receptor subsystem

In total there are 21 uncertain parameters. Groups u and v contain 6 parameters each and group w contains the remaining 9 parameters. The parameters assigned to each group are shown in table 5.1.

Parameter	Reaction	Group
k_1	Association rate of first insulin molecule to IR	u
k_{-1}	Dissociation rate of first insulin molecule from IR	u
k_2	Association rate of second insulin molecule to IR	u
k_{-2}	Dissociation rate of second insulin molecule from IR	u
k_3	Phosphorylation rate of surface IR	u
k_{-3}	Dephosphorylation rate of surface IR	u
k_4	Endocytosis of free IR	v
k_{-4}	Exocytosis of free IR	v
$k_4^{'}$	Endocytosis of bound IR	v
$k_{-4}^{'}$	Exocytosis of bound IR	v
k_{-5}	IR degradation	v
k_6	Dephosphorylation of intracellular IR	v
k_7	Phosphorylation of IRS	w
k_{-7}	Dephosphorylation of IRS	w
k_8	Formation of IRS/PI3K complex	w
k_{-8}	Separation of IRS/PI3K complex	w
k_{9stim}	Maximal conversion of PI(4,5)P2 to PI(3,4,5)P3	w
k_{11d}	Maximal phosphorylation of Akt	w
k_{-11}	Dephosphorylation of Akt	w
k_{15d}	Maximal phosphorylation of GSK3	w
k_{-15}	Dephosphorylation of GSK3	w

Table 5.1: Grouping of parameters in the insulin model.

The Morris method was used to measure the sensitivity of the GSK3 output to each of the three groups of parameters. As in the individual parameter analysis of the insulin model described in chapter 4 each parameter was allowed to vary in the range $\pm 50\%$ of its nominal value and an external insulin input of magnitude $1 \times 10^{-6} M$ was used. The overall Morris measure, which describes the sensitivity of the entire GSK3 output trajectory, was calculated for each group of parameters (see chapter 4 for a discussion of overall sensitivities). Five replicates of the analysis were performed to check the reproducibility of the method at the given sample size. Figure 5.1 shows the results of the analysis. The results are displayed as a bar chart and are also tabulated to show the values for $\mu_O^*(v)$ which can not be read from the plot. They show that the system-level output is most sensitive to uncertainty in the post-receptor signalling sub-model while uncertainty in the receptor recycling component is largely insignificant. This is consistent with the view that post receptor mechanisms represent the primary sites leading to disruption of the insulin signalling process (Pessin and Saltiel, 2000).



	$\mu_O^*(u)$	$\mu_O^*(v)$	$\mu_O^*(w)$
1	2.048	0.061	8.043
2	2.449	0.045	9.452
3	2.049	0.078	12.21
4	2.712	0.068	8.849
5	1.922	0.059	11.75

Figure 5.1: Results of applying the group Morris method to the insulin component model ($r = 20$, $p = 8$). The parameters are arranged into three groups associated with the three sub-system models: u = Parameters in the ligand-receptor binding sub-system, v = Parameters in the receptor recycling subsystem, w = Parameters in the post receptor subsystem. Five repetitions were performed. The groups were consistently ranked w more important than u more important than v. The table shows the numerical values which indicate that group v is largely insignificant.

In chapter 4 sensitivity analysis was performed on the individual parameters of the insulin model using both the Morris and Sobol methods. This analysis identified 10 influential parameters. One of these is involved in receptor binding and ranked 8th (in descending order of importance). The remaining 9 are involved in post receptor steps. None of the influential parameters are involved in the recycling process. (See chapter 4, figures 4.3, 4.12, 4.13 and 4.14 and section 4.3.2 for a discussion of these results). Based on this sensitivity analysis of individual parameters we would expect to find $\mu_O^*(w) \gg \mu_O^*(u) \gg \mu_O^*(v)$, indicating that group w is more important than u which in turn is more important than group v, and would also expect $\mu_O^*(v)$ to be approximately zero, indicating that it is largely insignificant. The results shown in figure 5.1 are consistent with these predictions.

These results demonstrate the potential benefits of using a group approach to study composite models. Firstly the approach is more economical than an individual parameter analysis. The Morris method requires $r_k(k+1)$ model evaluations to calculate a set of sensitivity measures for a model with k parameters and $r_G(G+1)$ runs when these are assigned to G groups (the subscripts k and G indicate that we may use different values of r for individual and group based analysis). In the case of the insulin model ($k = 21, r_k = 20$ and $G = 3, r_G = 20$) analysing groups of inputs requires

80 model runs compared with 440 for the individual parameter analysis. Generally, provided:

$$r_G < r_k \frac{(k+1)}{(G+1)} \tag{5.4}$$

the group approach will require fewer model runs.

More importantly in the analysis of a composite model a group approach gives us information about how uncertainty in the different component models, rather than the individual parameters, influence the system behaviour. This information can be useful in understanding the behaviour of the system, suggesting which sub-processes are most important in driving the system level behaviour. It is also useful for model development, suggesting which component models we should try to refine because they are important or those which could be simplified because uncertainty in their output has little effect on the system level behaviour. In the case of the insulin model, the group analysis suggests that we should focus on the post-receptor signalling component as it is the uncertainty in this sub-model output which is most important in determining the system level behaviour.

This benefit of a group approach could also been seen as a limitation. By focusing on groups of parameters we lose information about the importance of individual parameters. We can overcome this problem by combining an initial group based analysis with an individual parameter study. Performing a group analysis first has another potential benefit. If the group analysis identifies a component model as being insignificant we can exclude the parameters of that model from further analysis. For example, the insulin model analysis shows that we could leave the parameters of the receptor recycling sub-system out of any individual level analysis. This reduces the computational cost of the individual analysis and simplifies the amount of sensitivity information we need to process.

The following section describes the use of sensitivity analysis to look at the importance of individual parameters both within and across component models and discusses how we can use this information to help understand the behaviour of the system.

5.3 Intra and Inter-Sensitivity Analysis

As mentioned in the introduction the majority of applications of sensitivity analysis in biological modelling have focussed on models of a single component or scale. A small number of studies have considered multi-scale or multi-component models. Wang et al. (2008) applied sensitivity analysis to a multi-scale model of lung cancer. They used local SA techniques to look at the effects of

uncertainties in the molecular level parameters on the cellular level behaviour. They referred to this as cross-scale sensitivity analysis. Marino et al. (2008) considered the general case of a model with variables at different scales (for example molecular and cellular) or in different compartments (e.g. different organs). They defined the terms intra and inter-scale (compartment) sensitivity analysis to describe studies looking at the effects of parameters on outputs at the same or different scales (compartments) respectively. They demonstrated these concepts on a two compartment model of tuberculosis infection.

More generally, for a composite model consisting of multiple component models (which may represent processes at different scales) we can use the terms intra-sensitivities to refer to the sensitivity of an output variable to parameters of the same sub-model (i.e within a component) and inter-sensitivities to refer to the sensitivity of a variable to parameters of a different sub-model (across components). This section shows how these concepts can be used to investigate the behaviour of a composite biological model by combining them with the PCA based sensitivity analysis approach presented in chapter 4. The basic idea is outlined below.

Consider the case of a two component model consisting of model A and model B. The output of model A, $\mathbf{Y}(A)$, is the input to model B. Sensitivity analysis is performed on the composite model allowing the uncertain parameters of both sub-models to vary. The sensitivities of both model outputs to each parameter are calculated using the PCA based approach. The intra-sensitivities of model A tell us how its output depends on its own parameters while the inter-sensitivities of model B tell us how its output, $\mathbf{Y}(B)$, depends on the parameters of model A. Comparing these sensitivity measures can tell us if and how the effects of a perturbation in a component model parameter are propagated through the system. Assume that the output of model A is oscillatory and that its period and amplitude vary due to uncertainty in the model parameters. If parameter k_{A1} effects the amplitude of $\mathbf{Y}(\mathbf{A})$ and also effects $\mathbf{Y}(B)$ then we can infer that the amplitude of $\mathbf{Y}(A)$ is important in determining the behaviour of model B. Conversely, if parameter k_{A2} effects the period of $\mathbf{Y}(A)$ but has no effect on $\mathbf{Y}(B)$ this suggests that the period of A is not important. This information may be useful in helping us understand the system function and also in suggesting potential interventions. For example in the system described above we may expect other perturbations which affect the amplitude of $\mathbf{Y}(A)$ to have similar effects on the output of model B as perturbing k_{A1}. The following section demonstrates this approach on a composite biological model.

It is important to note that looking at multiple outputs does not significantly increase the computational demands of a given sensitivity analysis technique. We can use the same set of model runs to look at any number of model outputs. The only extra cost is in the additional data

processing required to calculate the sensitivity measures and in the extra memory requirements to store the additional output data.

5.3.1 A Two Component Example

The use of intra and inter-sensitivities is demonstrated on a two component system consisting of the glucagon receptor and calcium models. The system, shown schematically in figure 5.2, describes the change in the cytoplasmic calcium concentration caused by binding of glucagon to cell surface receptors. Details of the individual models are given in chapter 3. This system contains many of the typical features we may expect to find in composite biological models including non-linear terms and feedback loops and therefore is a good example to investigate the potential of the approach.

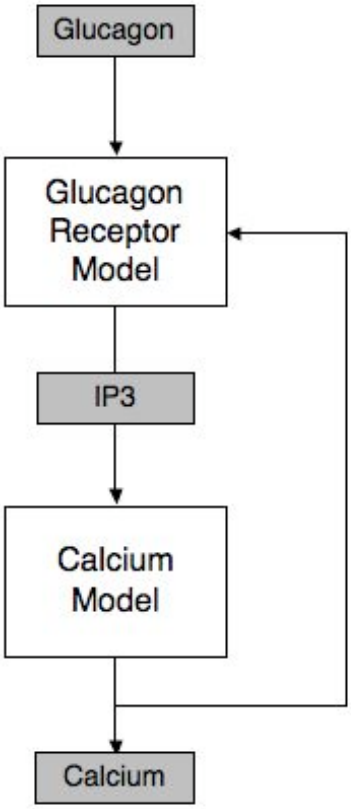


Figure 5.2: Schematic of the two component model combining the glucagon receptor and calcium sub-models. The receptor sub-model produces an output of IP3 (assumed to be proportional to the PLC concentration) which is passed as an input to the calcium sub-model. The calcium model describes the dynamics of the concentration of free intracellular calcium. There is also a negative feedback mechanism in which calcium inactivates active G-proteins, inhibiting the production of IP3 via PLC. The external input to the system is the concentration of glucagon.

The output of the composite model at the nominal parameter values in response to a sustained glucagon input of 0.2μM ($200nM$) introduced at $t = 20$s is shown in figure 5.3. This replicates the experimental conditions studied in Hansen et al. (1998) where glucagon was shown to produce a similar transient rise in intracellular calcium in hamster kidney cells expressing human glucagon receptors. Mine et al. (1993) also measured similar transient spikes in calcium in isolated rat hepatocytes in response to nM concentrations of glucagon.

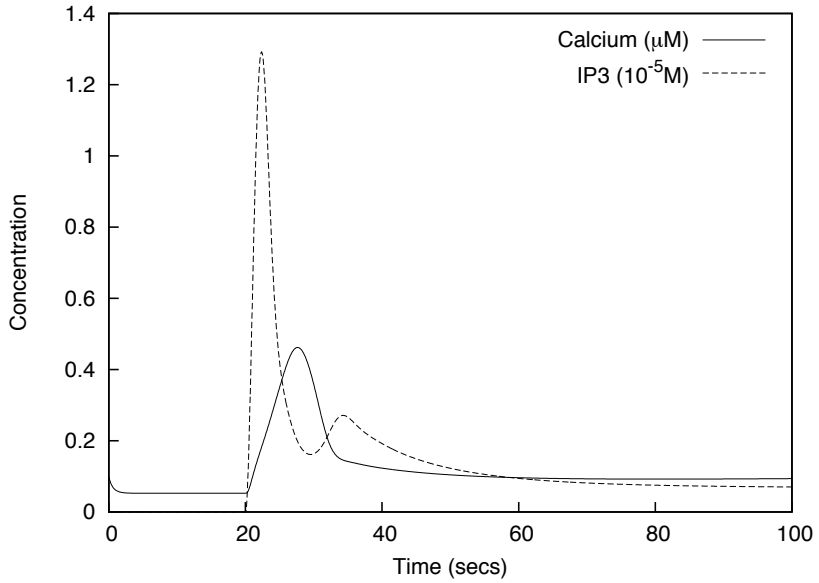


Figure 5.3: Output of the two component model at the nominal parameter values in response to an external glucagon input of 0.2μM at $t = 20$s.

Sensitivity analysis of the model was performed using the Morris method coupled with PCA. This screening design was introduced in chapter 4 and shown to produce results consistent with the variance based method of Sobol at a greatly reduced computational cost. In total 35 parameters were allowed to vary in the analysis (18 parameters from the glucagon receptor model, 17 from the calcium model).

Results

Sensitivity of the Calcium Output

First we consider how the calcium concentration depends on the parameters of the two sub-models. The overall Morris measure, defined in chapter 4 (see equation 4.34), is used to measure the effect of parameters on the entire output. The left hand side of figure 5.4 shows the sensitivity to the glucagon receptor sub-model parameters, the right hand side shows the sensitivities to the calcium sub-model parameters.

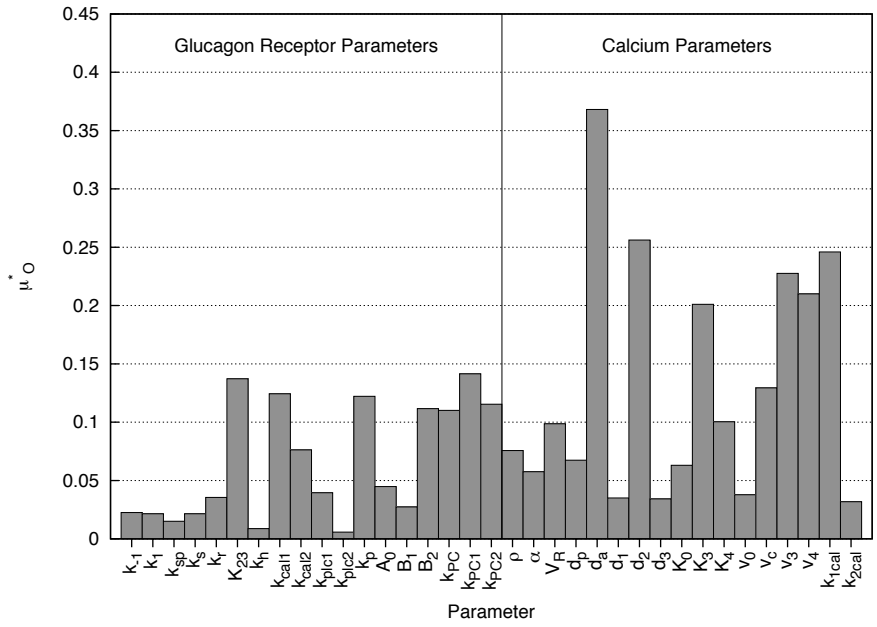


Figure 5.4: The overall sensitivities of the two component model calcium output computed using the Morris method. The left hand side of the figure shows the sensitivities to the glucagon receptor model parameters (inter-sensitivities) and the right hand side shows those of the calcium model parameters (intra-sensitivities).

The parameters of the calcium model which have the largest influence on its output are (in decreasing order of importance) d_a, d_2, k_{1cal}, v_3, v_4, K_3 and v_c. Of these, the three most important are related to the release of calcium from the endoplasmic reticulum (ER). This release is triggered by the binding of IP3 to receptors on the ER (IP3R) which are further stimulated by increasing calcium, causing so called calcium induced calcium release (CICR). At higher calcium concentrations calcium inhibits IP3R preventing calcium release. d_a is the threshold for calcium induced calcium release (CICR), d_2 is one of three parameters governing the inhibition of the IP3R by calcium and k_{1cal} is the maximal rate of IP3R meditated calcium release. These results are consistent with the view that calcium release from the ER plays a major role in producing calcium oscillations (Marhl et al., 2000). This is supported by evidence from various cell types that inhibition of IP3R (by heparin) blocks calcium oscillations (Carroll and Swann, 1992; Nett et al., 2002).

The calcium output is also sensitive to the parameters of the ER pump term, K_3 and v_3, which describe the uptake of calcium by the endoplasmic reticulum calcium ATPase (SERCA). This supports experimental evidence that addition of thapsigargin which inhibits SERCA activity disrupts intracellular calcium dynamics (Aguado et al., 2002) by causing depletion of the ER store of calcium. The influx and efflux of calcium from the extracellular medium is also important in determining the calcium output as shown by the sensitivities of v_c, the maximal rate of influx,

and v_4 the plasma membrane pump rate. This suggests that the presence of calcium in the extracellular medium and its movement into and out of the cell is important in producing the calcium dynamics. While it has been shown that calcium oscillations occur in cells in calcium-free medium the maintenance of these oscillations requires extracellular calcium (Visegrady et al., 2000). It is likely that an influx of calcium is required to replenish calcium in the ER and sustain the oscillations (Jones et al., 2008).

The calcium concentration is also shown to be sensitive, although to a lesser extent, to the parameters of the glucagon receptor model. The parameters which have most effect are K_{23}, k_{cal1}, k_p, B_2, k_{PC}, k_{PC1} and k_{PC2}. The effect of these parameters on the calcium model output is propagated via their effect on IP3, the variable which links the two models. Therefore by looking at the effect of these parameters on the modes of variation in the IP3 output we can draw some conclusions about the behaviours of the IP3 output which are important in determining the calcium output.

Sensitivity of the IP3 Output

The overall sensitivities of the IP3 output to the glucagon sub-model parameters are shown in figure 5.5. The IP3 sensitivity ranking is largely the same as that obtained for the stand alone glucagon receptor model (see section 4.4.2). The most important parameters in determining the IP3 output are K_{23}, k_{plc1}, k_p and B_2. The only significant difference is the increased importance of k_{cal1} and k_{cal2}, the parameters describing the negative feedback of calcium on active G-proteins. This is due to the presence of a non-zero calcium concentration when the model is coupled with the calcium model (in the analysis of the individual glucagon receptor model calcium was set at zero).

Propagation of Uncertainty

Figure 5.5 also shows the sensitivities of the calcium output to the IP3 parameters (these have already been discussed above). By comparing these we can draw some conclusions about the function of the complete system. One observation of interest is that k_{plc1} which is the second most important parameter in terms of the IP3 output is only the 10th most important in terms of its effect on the calcium output. Looking at the results for each principal component, it is apparent that the high overall ranking of k_{plc1} is primarily due to its high score on PC1. Figure 5.6 shows the first principal component, its effect on the mean model output and the values of μ^*.

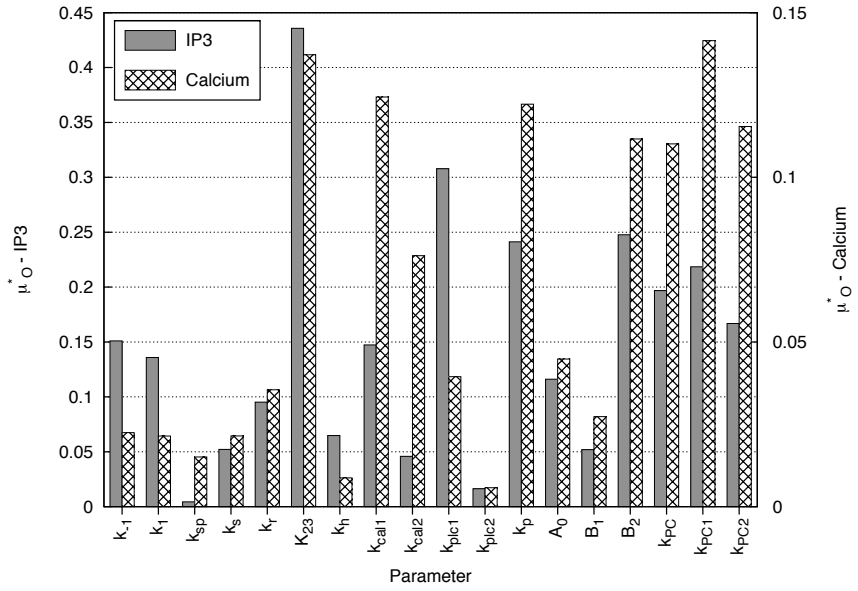
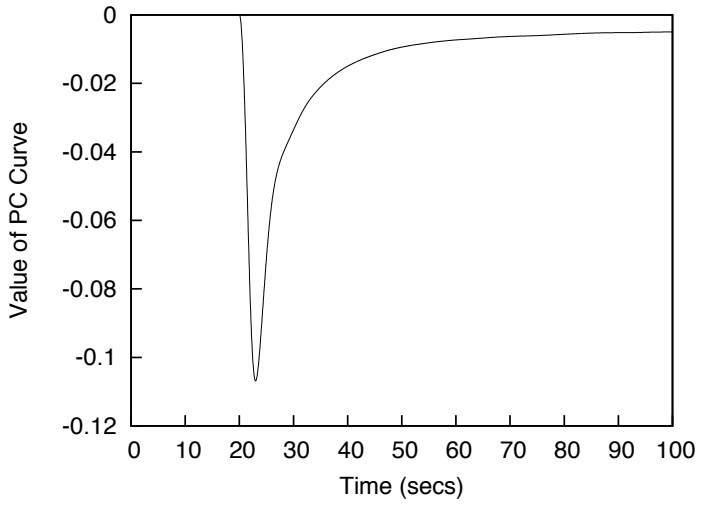
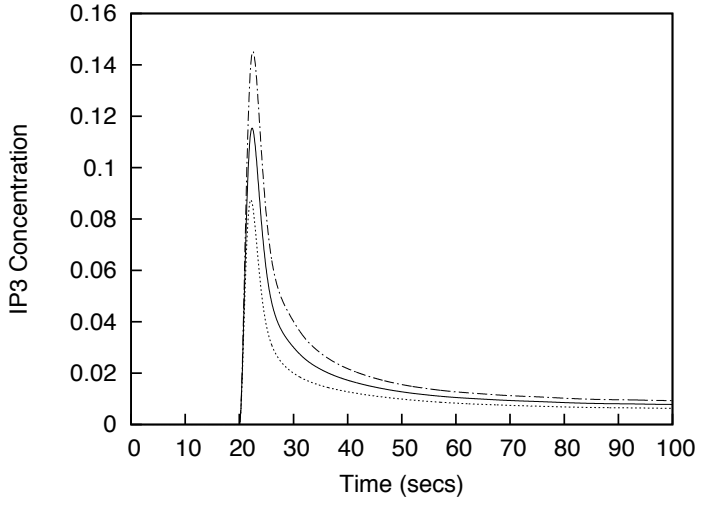


Figure 5.5: The overall sensitivities of the two component model IP3 and calcium outputs to the glucagon receptor model parameters. Sensitivities are calculated using the Morris Method.

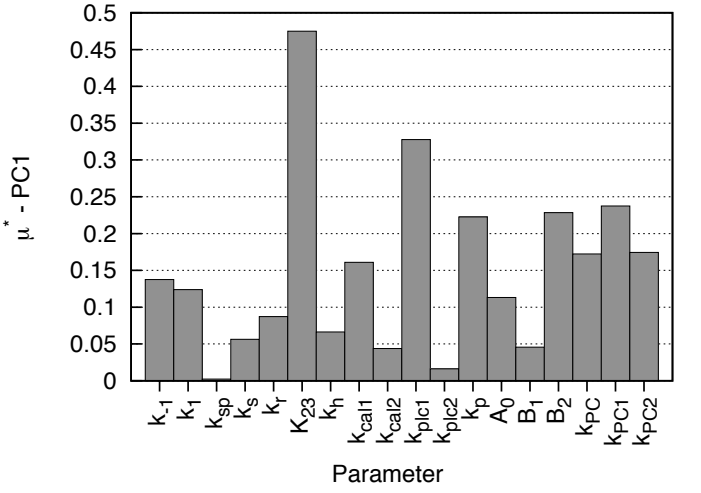
Figure 5.6 shows that the first PC describes variation in the magnitude of the IP3 output. Similarly the most important type of variation in the calcium output is uncertainty in the magnitude of the response (not shown). One explanation for the lack of effect of k_{plc1} on the calcium output is therefore that the magnitude of the IP3 concentration does not effect the magnitude of the calcium response. This is consistent with the behaviour of the standalone calcium model. Hofer (1999) showed in his original paper (from which the calcium model is taken) that the amplitude of the calcium oscillations produced by the model are largely independent of the IP3 dose over a large range of values. The other parameter which is very important in determining the variation in PC1 is K_{23}. In contrast to k_{plc1} this parameter is important in terms of the calcium output (see figure 5.5). This can be explained by its higher scores on PC2 and PC3 of the IP3 output when compared to k_{plc1}.



(a)



(b)



(c)

Figure 5.6: Panel a shows the first principal component of the IP3 output of the two component model. Panel b shows the effect of this model of variation on the mean IP3 output. Panel c shows the sensitivity of this mode of variation to the glucagon receptor component model parameters.

5.4 Discussion and Conclusions

Biological systems typically consist of sub-systems which operate at different scales (cellular vs molecular) or take place in different compartments or organs. Mathematical models are a powerful way to investigate such systems allowing us to combine information from different levels into a complete system. One approach to the construction of such models is to connect together models of the various components to produce a composite model of the entire system. This chapter has discussed approaches for performing sensitivity analysis of such models. These approaches make use of the PCA based SA methods described in chapter 4.

Two techniques have been presented. The first uses the concept of group SA to investigate the importance of the various component models on the behaviour of the composite model. It was shown that the Morris method could be used to perform group sensitivity analysis using a standard test case. The method was then applied to the insulin model, treating it as a composite model comprising of receptor binding, receptor recycling and post-receptor signalling component models. The results indicated that the post-receptor signalling component is most important and that the receptor recycling sub-system is insignificant in producing variation in the system level output. These results were consistent with the findings of our previous individual parameter level analysis.

The group approach allows us to identify where we should focus future modelling efforts (refining the description of the post receptor signalling pathway). The use of group analysis is also very economical, requiring fewer model runs than an individual parameter analysis. In the analysis of the insulin model the $k = 21$ parameters were combined into $G = 3$ groups reducing the number of model runs from $r(k+1) = 440$ to $r(G+1) = 80$. It may also allow us to reduce the computational demands of future analysis by identifying the groups of parameters which we could exclude from further analysis (in the case of the insulin model the 6 parameters of the receptor recycling sub-system could be fixed at their nominal values).

The second half of this chapter has discussed the application of individual parameter level analysis to composite models. The concepts of intra and inter sensitivities were introduced and it was suggested how we could use these ideas to investigate the effects of parameters both within and across components. The idea was demonstrated on a two component model consisting of the glucagon receptor and calcium models described in chapter 3. By using the principal component analysis approach and comparing the effects of parameters on their own component model output and the output of other components we can try to understand how uncertainty in a parameter is propagated through the system. For example, analysis of the two component example suggested

that uncertainty in the magnitude of the glucagon receptor model output (IP3) had a limited effect on the calcium dynamics. This type of information may be useful in understanding the function of the system and in suggesting potential therapeutic targets or interventions.

It is suggested that these two approaches can be combined to provide an efficient methodology for the analysis of composite models. First a group analysis is performed to identify the important component models and suggest any parameters which can be excluded from further analysis. This is followed by an individual parameter level analysis in which both intra and inter sensitivities are considered allowing us to investigate the mechanisms by which parameter uncertainties are propagated through the system. The next chapter presents the application of this methodology to the composite model of glucose homeostasis described in chapter 3.

Chapter 6

Sensitivity Analysis of a Composite Model of Blood Glucose Regulation

This chapter presents an application of the sensitivity analysis approaches discussed in chapters 4 and 5 to the composite model of glucose homeostasis presented in chapter 3. The results of the analysis suggest a number of hypotheses about the function of the glucose homeostasis system and identify future directions for the development of the model.

6.1 Introduction

In this section the methods developed in chapters 4 and 5 are applied to the composite model of glucose regulation described in chapter 3. The model consists of seven component models representing various aspects of the biology:

- Pancreas Model - describes the production of glucagon and insulin by the pancreas as a function of the blood glucose concentration
- Glucagon Receptor Model - describes the activation of G_q protein coupled receptors by glucagon and the subsequent activation of IP3
- Calcium Model - describes the IP3 dependent intracellular calcium dynamics
- cAMP Model - describes the activation of G_s protein coupled receptors by glucagon and the subsequent activation of PKA in a cAMP dependent manner

- Insulin Model - describes the signalling pathway initiated by binding of insulin to cell surface receptors resulting in inactivation of GSK3
- Blood Model - describes the transport of glucose between the blood and the liver
- Glycogenolysis Model - describes the synthesis and breakdown on glycogen by the liver in response to glucose levels, GSK3, calcium and PKA

When connected together these models describe the response to an external input of glucose or an increased demand for glucose by the body. The model behaviour has previously been explored at the nominal parameter point in response to different external challenges and it was shown that the model is able to reproduce qualitative experimental observations. In addition the effects of varying individual parameters, namely the sensitivity of the glycogenolysis model to insulin, have been studied (Hetherington et al., 2009).

By performing a more detailed sensitivity analysis we hope to increase our understanding of the system function. The results of the analysis will provide information on how the different components of the system control the output. This may suggest new avenues for research into the regulation of blood glucose levels. In addition it will help us focus modelling effort on the most relevant parts of the model to improve its performance and utility.

The analysis presented in this chapter is separated into four stages:

1. Examine the behaviour of the model at the nominal parameter values
2. Define distributions for the uncertain parameters
3. Perform a group SA
4. Perform an individual parameter SA

First we examine the behaviour of the model at the nominal parameter values. We then need to define distributions for the uncertain parameters. The third step is to perform a group sensitivity analysis, treating the parameters of each of the seven component models as a separate group. This analysis will allow us to investigate which component models are most important in driving the system level behaviour and also to identify any sub-models which are insignificant. The parameters of these unimportant models can be excluded from further analysis.

The final stage is to perform an individual parameter level analysis using the PCA-based Morris method. Using this method we can identify the most important individual parameters and explore the ways in which uncertainty in sub-model parameters influences the system level behaviour.

6.2 Behaviour of the Model at the Nominal Parameter Point

Before performing any sensitivity analysis it is useful to study the behaviour of the model at the nominal parameter values and to see how this behaviour depends on the external glucose concentration driving function. A variety of different input functions could be considered. In this chapter we will focus on the response to continuous glucose inputs. For positive values this is intended to represent the conditions of continuous enteral nutrition (delivery of a nutritionally complete diet directly into the stomach) or continuous glucose infusion which have both been used to study the response in humans (Kraegen et al., 1972; Simon et al., 1987). Mathematically this is represented as a step function $M(t) = M$, a constant (units of mM/s), for $t \geq 500s$.

Figure 6.1 shows the output of the model at the nominal parameter point for a range of glucose values. Four different types of behaviour are displayed. For negative glucose inputs blood glucose falls from its initial state and is stabilised at a lower value by the release of glucose from the liver. Once the glycogen stores in the liver have been depleted the blood glucose level falls to zero at a rate determined by the external input. For low positive inputs ($M = 5$) blood glucose rises to an elevated stable level. For intermediate inputs ($M = 15$) the glucose level also rises to a new elevated steady state but first "overshoots" this value. For high positive inputs ($M = 25$) the model is unable to regulate the blood glucose level which rises without limit. This is because glucose can not be converted into glycogen at a sufficiently fast rate to accommodate the external input.

Both the rise without limit and the decay to zero are probably non-physiological behaviours. The model only describes the glucose $\rightleftharpoons$ glycogen inter-conversion and does not include processes which would take place under these extreme circumstances. At low glucose levels gluconeogenesis (the production of new glucose from lactate, glycerol and amino acids) would help limit the onset of hypoglycemia (Landau et al., 1996). At high glucose levels excess glucose would be diverted into a number of other pathways which are not included in the current model. These include increased uptake by other tissues including skeletal muscle and the kidney (Meyer et al., 1998), increased synthesis of fatty acids by the liver (Postic and Girard, 2008) and the excretion of excess glucose into the urine (Kaneko et al., 1978).

In the rest of this chapter sensitivity analysis is applied to the model to see how the model output is affected by uncertainty in the nominal parameter values. The first step in applying the analysis is to define uncertainty distributions for the model parameters. This task is discussed in the next section.

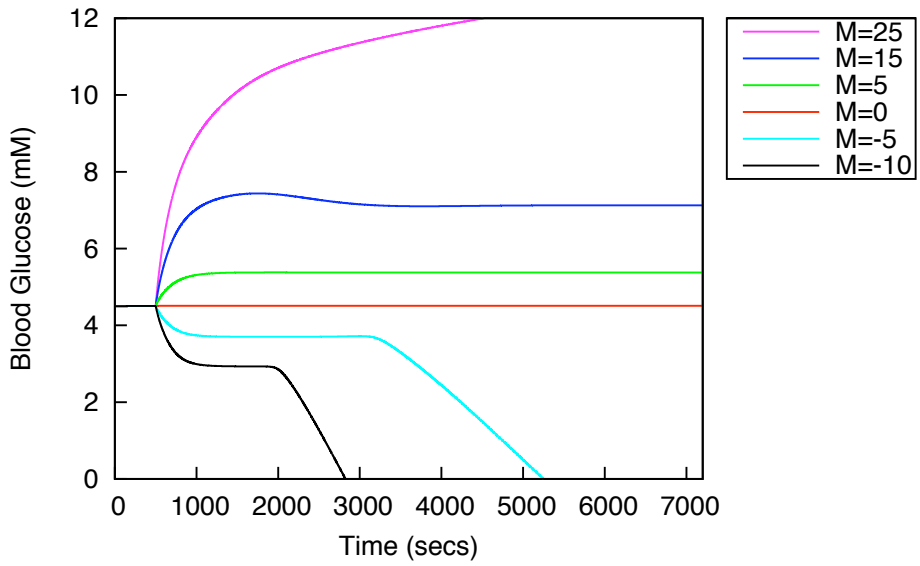


Figure 6.1: The output of the composite model at the nominal parameter values for different external glucose inputs.

6.3 Defining Parameter Distributions

The selection of appropriate parameter distributions is an important part of global SA methods because the results of the analysis may be dependent on the choices (Lipton et al., 1995). It can also be the most difficult and time consuming stage of performing the analysis (Saltelli et al., 2000a). The choice of distribution is often governed by the availability of data.

A framework for determining parameter distributions for MC simulations based on available data was proposed by Lipton et al. (1995). If the specific distribution type of a parameter is known (for example a normal distribution) we can try to estimate the parameters of that distribution (for example the mean and variance) using the available data. If the distribution is not known we can try to select a class of distributions based on our knowledge of the parameter, for example is it continuous or discrete and are there bounds on its possible values? We can then use goodness-of-fit (GOF) techniques to identify the most likely distribution and then estimate the parameters of that distribution as described above. If these steps are not successful bootstrapping techniques (Davison and Hinkley, 1997) can be used to try to generate a distribution from the available data. If bootstrapping techniques can not be used due to the small number of available data values then uniform distributions between the minimum and maximum values can be used. This process is shown in the flow diagram in figure 6.2. In many fields where "hard data" is not typically available "expert opinion" (Cooke, 1991) is often used to define uncertainty distributions (Clemen and Winkler, 1999).

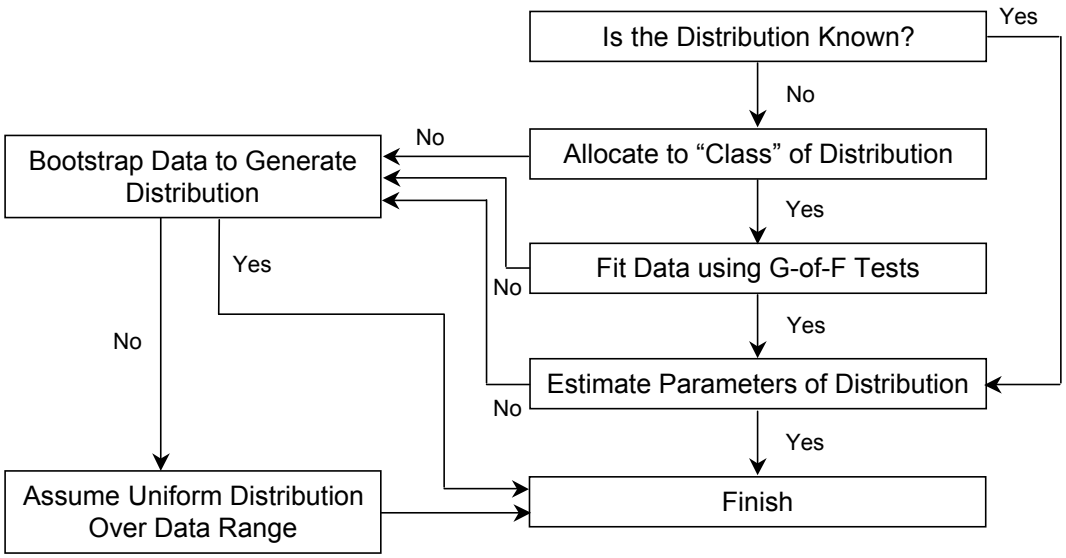


Figure 6.2: Framework for selecting input distributions in Monte Carlo simulations (Based on Figure 1 in Lipton et al. (1995)).

The purpose of the analysis is also important when selecting the parameter distributions. In the case of a biological or physiological system a sensitivity analysis may have a number of different aims. If we are interested in understanding the behaviour of the system under normal conditions we need to select ranges which represent the variation in the parameters observed in normal subjects. Alternatively we may wish to investigate the important parameters in a particular disease state or condition. In this case we should extend the ranges to include plausible values associated with the condition of interest.

More generally we may be interested in investigating the parameters which the model output is sensitive to. For example we may wish to identify potential targets via which we may influence the system output. In this type of analysis the uncertainty distributions need not be based on the experimentally observed uncertainty in the model parameters. For example we may include parameters whose value is not regarded as uncertain to investigate the potential effects of artificially perturbing those parts of the system. A convenient form of input distribution in these cases is to adopt uniform ranges based on a percentage of the nominal parameter values. This is the approach followed in this chapter where the main aim is to demonstrate the potential of the methodology developed in chapters 4 and 5.

6.4 Group Sensitivity Analysis

This section describes the application of a group level sensitivity analysis to the composite model. The parameters are grouped by component model. This allows us to investigate which component models are most important in driving the system level behaviour and identify any component models which are insignificant.

The composite model contains seven component models giving us seven groups of parameters: glycogenolysis (9), blood (2), pancreas (7), insulin (21), cAMP (13), glucagon receptor (18) and calcium (17) (numbers in brackets indicate the number of parameters in each model/group).

The Morris method by groups was applied to the composite model for different external glucose inputs ranging from $M = -25$ to $M = 25$ in steps of 2.5. The model is solved from $t = 0$ to $t = 7200s$ (2 hours).

Values of $r = 30$ and $p = 10$ were used. These were shown to produce consistent rankings of the component models in replicates of the analysis. Figure 6.3 shows the overall sensitivity (μ^*_O) of the blood glucose concentration to each of the component models as a function of the magnitude of the external glucose function. The data points show individual replicate values and the line shows the average across all three replicates.

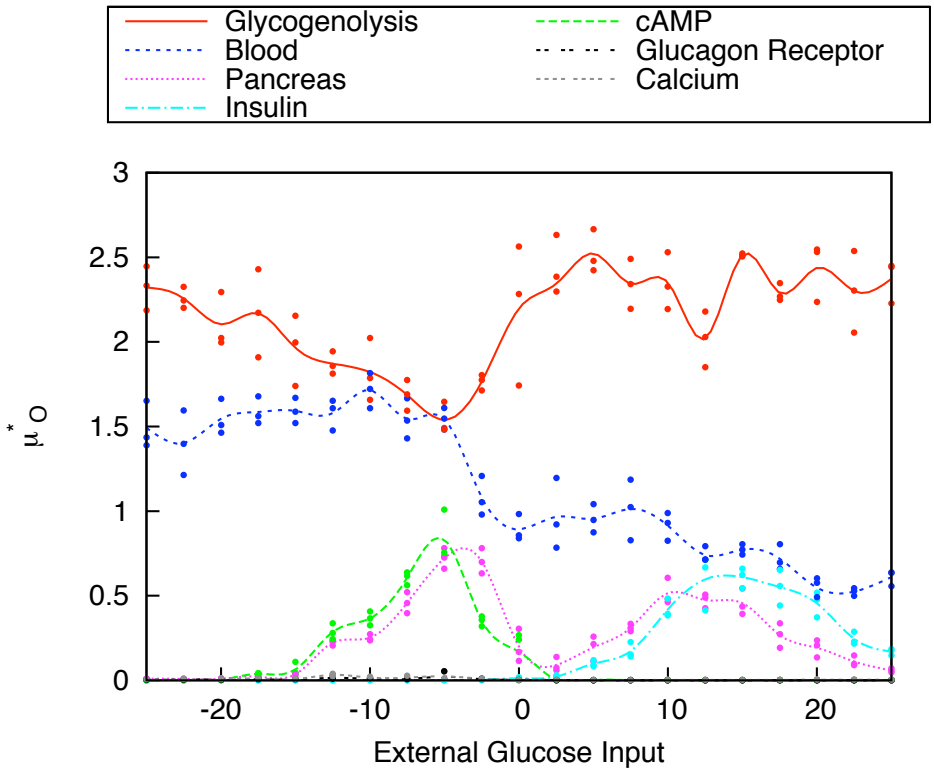


Figure 6.3: The results of applying a group sensitivity analysis to the composite model using the Morris method. Parameters were grouped by component model. The overall Morris measure is displayed as a function of the external glucose input. Data points show the results for three replicates of the analysis, lines show the average sensitivity calculated over all replicates.

The results show that the sensitivities depend strongly on the magnitude of the glucose input. In particular there is a clear divide between the results for positive and negative inputs. The results for the different component models are discussed below.

6.4.1 Glucagon Receptor and Calcium Models

The glucagon and calcium sub-models are shown to be unimportant for all external input values. This is unsurprising for positive values where blood glucose would not be expected to fall sufficiently below the threshold for production of glucagon by the pancreas. Under these conditions the glucagon signalling pathways will not be activated meaning the release of calcium from the ER will not be triggered. The lack of importance for negative inputs is of more interest. This indicates that even when glucagon is produced uncertainty in the glucagon receptor and calcium models does not affect the blood glucose dynamics. This suggests that calcium plays a minor role in the regulation of glycogen metabolism.

There is debate regarding the physiological importance of glucagon dependent increases in cytosolic calcium (Exton, 2001; Aromataris et al., 2006). The results of the sensitivity analysis support the view that its role in glycogenolysis is minor. This is consistent with a number of experimental studies including Pittner and Spitzer (1993) who showed that inhibiting glucagon activation of PLC and IP3 does not affect the ability of the hormone to stimulate glycogen phosphorylase suggesting that activation of calcium in an IP3 dependent manner is not important for the regulation of glycogen metabolism.

6.4.2 Blood and Glycogenolysis Models

The blood and glycogenolysis models are found to be the two most important across all inputs. The glycogenolysis model is most important for positive inputs, the two models then converge to a point at approximately $M = -5$ where they are ranked approximately the same. They then diverge for more negative values with the glycogenolysis model becoming more important again.

The high sensitivity values for the glycogenolysis model imply that the synthesis and breakdown of glycogen are the most important parts of the system. This result is understandable, the amount of glucose being stored or released by the liver is crucial to determining the blood glucose level. The individual parameter analysis presented in section 6.5 will allow the importance of the different parts of this process to be investigated.

The importance of the blood model may be due to a number of different effects as a result of the connections between the component models (see figure 3.2). Firstly uncertainty in the blood model directly affects the amount of blood glucose via changes in the transport of glucose between the liver and the blood. Secondly uncertainty in the blood glucose concentration due to variations in the blood model parameters will affect the production of glucagon and insulin by the pancreas. This uncertainty will feedback onto the blood glucose concentration via the hormonal regulation of glycogen metabolism. Finally the importance of the blood model may in part be due to the auto-regulatory effect of blood glucose on glycogen metabolism. Autoregulation is known to play an important role in the maintenance of normoglycemia (Moore et al., 1998). In studies in human subjects hyperglycemia in the presence of sub-basal insulin and glucagon concentrations has been shown to reduce hepatic glucose production by $\sim 80\%$ (Sacca et al., 1978). The main effect appears to be inhibition of glycogenolysis with gluconeogenesis not significantly reduced (Rossetti et al., 1993). Hypoglycemia has also been shown to influence hepatic glucose production in a hormone independent manner (i.e. not via the increased production of glucagon by the pancreas). Experimental evidence also indicates that autoregulation is more important in the response to severe hypoglycemia than at higher blood glucose levels (Bolli et al., 1985). This is consistent

with the increase in importance of the blood model between $M = -2.5$ and $M = -5$ (for larger negative inputs lower blood glucose levels will be experienced). The individual parameter analysis presented in section 6.5 may shed light on which mechanisms are important.

6.4.3 The Pancreas, Insulin and cAMP Models

The importance of the pancreas, insulin and cAMP models are also dependent on the glucose input. For positive inputs the pancreas and insulin models are important and the cAMP model is insignificant. For negative inputs the situation is partially reversed with the pancreas and cAMP models identified as influential and the insulin model found to be unimportant.

Positive Inputs

For positive inputs the blood glucose concentration will typically remain above the threshold value below which the pancreas produces glucagon. This explains the lack of importance of uncertainty in the cAMP model for such inputs.

The sensitivity to both the pancreas and insulin models varies non-monotonically with the external glucose input. Both start at low values and increase with increasing glucose peaking between $M = 10$ and $M = 15$. Their importance then decreases for larger inputs.

At low positive inputs blood glucose will not regularly exceed the threshold at which the pancreas begins to produce insulin. This could explain why uncertainty in the pancreas model has little effect on the model behaviour. If only minimal amounts of insulin are present then uncertainty in the insulin model will also have a minimal effect on the system level behaviour.

As the glucose input increases, blood glucose will reach higher values and stimulate the production of more insulin by the pancreas model. The uncertainty in the pancreas model then becomes more important as it affects the amount of insulin produced. The increase in insulin means the insulin model is activated and uncertainty in the model becomes important, producing uncertainty in the level of active GSK3. This uncertainty is propagated to the glycogenolysis model affecting the rate of glycogen synthesis and the blood glucose level.

Beyond $M = 20$ the liver begins to be unable to cope with the external glucose input as the rate of glycogen synthesis reaches its maximum. At this point uncertainty in the active GSK3 concentration caused by uncertainty in the pancreas and insulin models is unimportant in controlling the glucose concentration which tends to increase without limit (see figure 6.1).

Negative Inputs

For negative inputs the pancreas model will produce little insulin meaning the insulin model will be unimportant as shown by the group analysis. The sensitivity to the pancreas and cAMP model display a similar pattern to that of the pancreas and insulin model for positive inputs. Both increase in importance with increasingly negative inputs peaking in importance at approximately $M = -5$. Their importance then decreases as M becomes more negative.

The behaviour can be explained in a similar way as that of the pancreas/insulin models. At small negative values little glucagon is produced. As a result neither the pancreas or the cAMP models is important. As the input becomes more negative the blood glucose level falls producing more glucagon and triggering the cAMP model. Both the pancreas and cAMP models increase in importance. At higher values the glycogen stores in the liver are rapidly emptied such that the effects of cAMP on glycogenolysis are less important.

6.4.4 Fixing Model Parameters

The next section presents an individual level parameter analysis of the blood glucose concentration. By studying the effects of individual parameters both within and across components it is hoped we will be able to gain a better understanding of the ways in which component level uncertainties influence the system level behaviour.

Before performing the analysis we can use the results of the group analysis to reduce the number of uncertain parameters in the analysis thus reducing the computational time required. The group sensitivity analysis results suggest that we can fix the 35 parameters of the glucagon receptor and calcium models for the purposes of the individual level sensitivity analysis. We can also fix the parameters of the cAMP and insulin models for certain external glucose inputs as these only appear to significantly influence the system level output for negative and positive inputs respectively. Figure 6.3 does show that there is possibly some overlap for small positive values at which the cAMP model has a minor effect. This is possibly the result of oscillations in the blood glucose concentration around the threshold value for the production of insulin or glucgaon. It is possible that a similar effect may be observed for the insulin model. To ensure we do not miss these possible effects the insulin and cAMP parameters will be included in the full analysis for values in the region $M > -5$ and $M < 5$ respectively.

6.5 Individual Parameter Sensitivity Analysis

An individual parameter analysis was applied to the composite model using the Morris method coupled with PCA. The analysis was performed for a range of external glucose inputs. A maximum of 52 parameters were allowed to vary in the analysis (the parameters varied in the group analysis with the exception of the glucagon receptor and calcium parameters which were fixed at their nominal values (see above)). For high positive inputs ($M > 5$) 39 parameters were analysed (the 13 parameters of the cAMP model were fixed to their nominal values). Similarly for large negative inputs the 21 parameters of the insulin model were fixed giving 31 uncertain parameters.

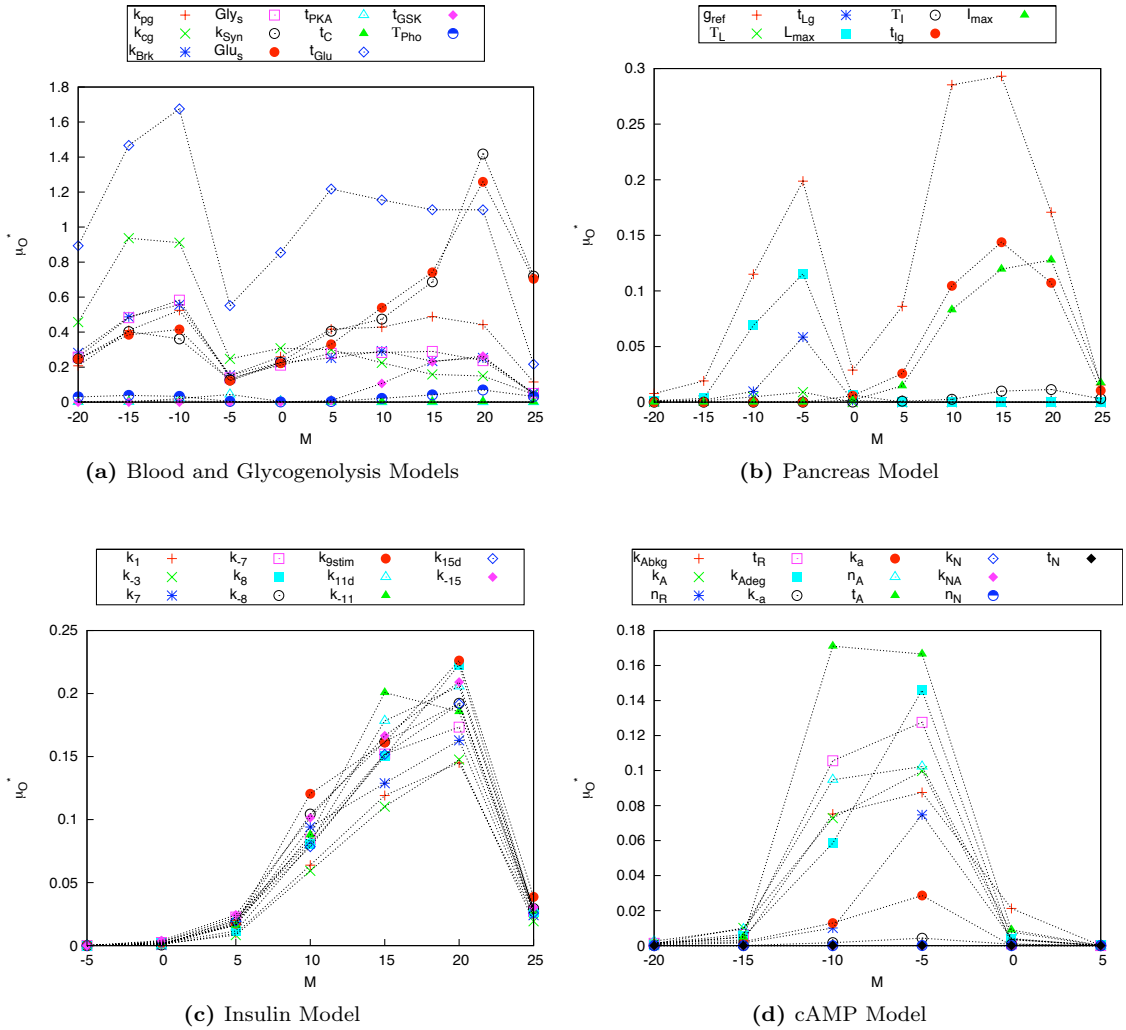
6.5.1 Overall Sensitivities

Figure 6.4 shows the overall sensitivities of the composite model output to individual parameters as a function of the external glucose input. The overall sensitivities measure the effect of a parameter on the entire model output. The results for the parameters of different component models are plotted separately to improve the presentation. It is clear that the most important parameters belong to the glycogenolysis and blood models while the important parameters in the other component models are ranked similarly (compare the y axis scales in figure 6.4). The interesting features of each component model are discussed below.

Blood and Glycogneolysis Models

Figure 6.4a shows the results for the blood and glycogenolysis models. The blood model which describes the transport of glucose between the liver and the blood consists of a passive transport term and an additional active influx term which is included to account for the selective transport of glucose but not $Glc-6-P$ out of the liver. As the total pseudo-glucose concentration (glucose and $Glc-6-P$) is represented as a single variable the efflux from the liver would be overestimated without the additional term. The individual parameter SA indicates that k_{pg}, the rate of active transport into the liver, is more important for positive glucose inputs while k_{cg}, the rate of passive transport along the glucose gradient, is more important for negative inputs. This result makes biological sense. When blood glucose levels are high (due to a positive external input) we would expect transport into the liver to be most important, when blood glucose is low (due to a negative input) we would expect the release of glucose from the liver to become important.

t_{Glu} is found to be the most important parameter in the glycogenolysis model. This parameter determines the threshold value around which glucose affects the activity of glycogen phosphorylase (GPho) and glycogen synthase (GSyn). This suggests that auto-regulation of hepatic glucose



(a) Blood and Glycogenolysis Models

(b) Pancreas Model

(c) Insulin Model

(d) cAMP Model

Figure 6.4: The overall sensitivities of the composite model calculated via the Morris method. Results are shown as a function of the external glucose input.

production by glucose is an important part of the control of blood glucose levels (Moore et al., 1998). The thresholds for active PKA (t_{PKA}) and GSK3 (t_{GSK}) are far less important. This could suggest that the affects of PKA and GSK3 on the activity of GPho and GSyn are not important. This seems unlikely given the importance of the pancreas, insulin and cAMP models. It is possible that the threshold values are less important because PKA and GSK3 display switch like behaviour and are typically well above or below the threshold values. Uncertainty in the threshold value may therefore have little influence on their affect on glycogen metabolism. The threshold for calcium to influence the metabolism of glycogen, t_C is insignificant. This is consistent with the conclusion from the group analysis that the activation of calcium by glucagon is not important in regulating glycogenolysis.

For positive inputs the parameters describing the maximal rate of synthesis of glycogen (k_{Syn} and Glu_s) are important because the liver will be converting large amounts of excess glucose into glycogen. As the external glucose input is reduced the importance of these parameters is reduced and the rate of glycogenolysis (k_{Brk} and Gly_s) becomes more important. For negative inputs glycogen breakdown is more important than glycogen synthesis.

Pancreas Model

Figure 6.4b shows the results for the pancreas model parameters. Two different groups of parameters are shown to be important depending on the input function. For positive values the most important parameters are g_{ref} (the blood glucose level above (below) which the pancreas produces insulin (glucagon)), t_{Ig} (the Hill function threshold for insulin production) and I_{max}, the maximum concentration of insulin produced. τ_I, the time-scale for insulin production, is much less important. This suggests that the amount of insulin produced by the pancreas has a greater effect on the overall variation in the blood glucose concentration than the speed of its release. As expected the parameters governing glucagon production are unimportant.

For negative inputs a similar pattern is observed with the parameters describing insulin and glucagon reversed. As for positive inputs g_{ref} is the most important parameter. This is followed by L_{max} and t_{Lg} with τ_L less important. Again this suggests that it is the amount of hormone produced rather than the time-scale of the production which is most important in determining the overall variation in the output. The parameters controlling insulin production are not important for negative inputs.

Insulin Model

The important parameters of the insulin model are the same as those identified via the analysis of the stand-alone model presented in chapter 4 (including k_1 which was shown to be important when the external input was varied (see section 4.4.4)). Figure 6.4c shows how the overall sensitivity to each of the 11 important parameters varies with the external glucose input (results for the 10 insignificant parameters are not shown). Each parameter shows the same qualitative variation; unimportant at negative and low positive values, increasing in importance with increasing glucose before falling again for high values. This variation is consistent with the group analysis results.

cAMP Model

Figure 6.4d shows the sensitivities of the blood glucose level to the cAMP model parameters. These display a similar variation with the external glucose input as the group sensitivity of the cAMP model (see figure 6.3); the importance of the individual parameters is low for positive and small negative inputs, increases for intermediate negative inputs then falls as the magnitude of the glucose input is increased further.

The model describes three processes: the production of cAMP, the activation of PKA and the nuclear localisation of active PKA. The parameters which govern nuclear localisation of active PKA (k_N, t_N, k_{NA} and n_N) are not important. This process was originally included to allow future extensions of the model to describe transcriptional level regulation. While it may play a role at the transcriptional level (Kawaguchi et al., 2001) the results of the analysis indicate that the nuclear localisation of PKA does not affect the short term regulation of blood glucose levels.

The most important parameters are involved in the production or degradation of cAMP (t_R and k_{Adeg}) or the activation of PKA (t_A). Both the production of cAMP and the activation of PKA are described by Hill function dynamics. Interestingly for both processes it is the threshold values (t_R and t_A) which are more important than the maximal rates (k_A and k_a). The rate of deactivation of PKA (k_{-a}) appears to be largely unimportant.

6.5.2 Principal Component Sensitivities

In this section the individual principal component based sensitivities are discussed. These results identify the main types of variation in the model output and allow us to investigate whether different parameters are important in producing the different types of variation.

Of particular interest is the role of the insulin signalling pathway in determining the model output. Insulin signalling is a key component in the regulation of blood glucose and defects in the

pathway are believed to be important in the onset of type 2 diabetes (Brady and Saltiel, 1999). Understanding how perturbations of this pathway affect the system output could potentially aid efforts to develop treatments for the condition. We will therefore focus on an external input value ($M = 15$) where the group and overall sensitivity analysis indicates that the insulin model is important

Principal Components

Figure 6.5 shows the first three principal components of the blood glucose concentration for an external input of $M = 15$. Panels a,c and e show the principal components, b,d and f show the mean model output plus (dotted line) and minus (dashed line) a multiple of each component.

The first principal component describes variation in the magnitude of the blood glucose concentration. Because the principal component is negative at all time-points, model runs with positive scores on this component will have a lower than average concentration while negative values will produce higher concentrations.

The main effect described by the second principal component is variation in the "overshoot" of the initial rise in blood glucose above its elevated steady state (see figure 6.1). Model runs with high scores for this component will have large transient rises in blood glucose.

PC3 describes the possible onset of oscillations in the blood glucose concentration. These oscillations have a period of approximately 50 minutes but are not sustained and decay to a single constant value. While not identical, these oscillations display a qualitative similarity to the oscillations which have been observed experimentally in blood glucose under a variety of conditions (Kraegen et al., 1972; Simon et al., 1987; Polonsky et al., 1988; Shapiro et al., 1988). Known as ultradian oscillations, these occur with periods of between $50 - 120$ minutes (Simon et al., 1987; Simon, 1998). Figure 6.6 shows an example of oscillations in blood glucose recorded in human subjects during oral glucose administration.

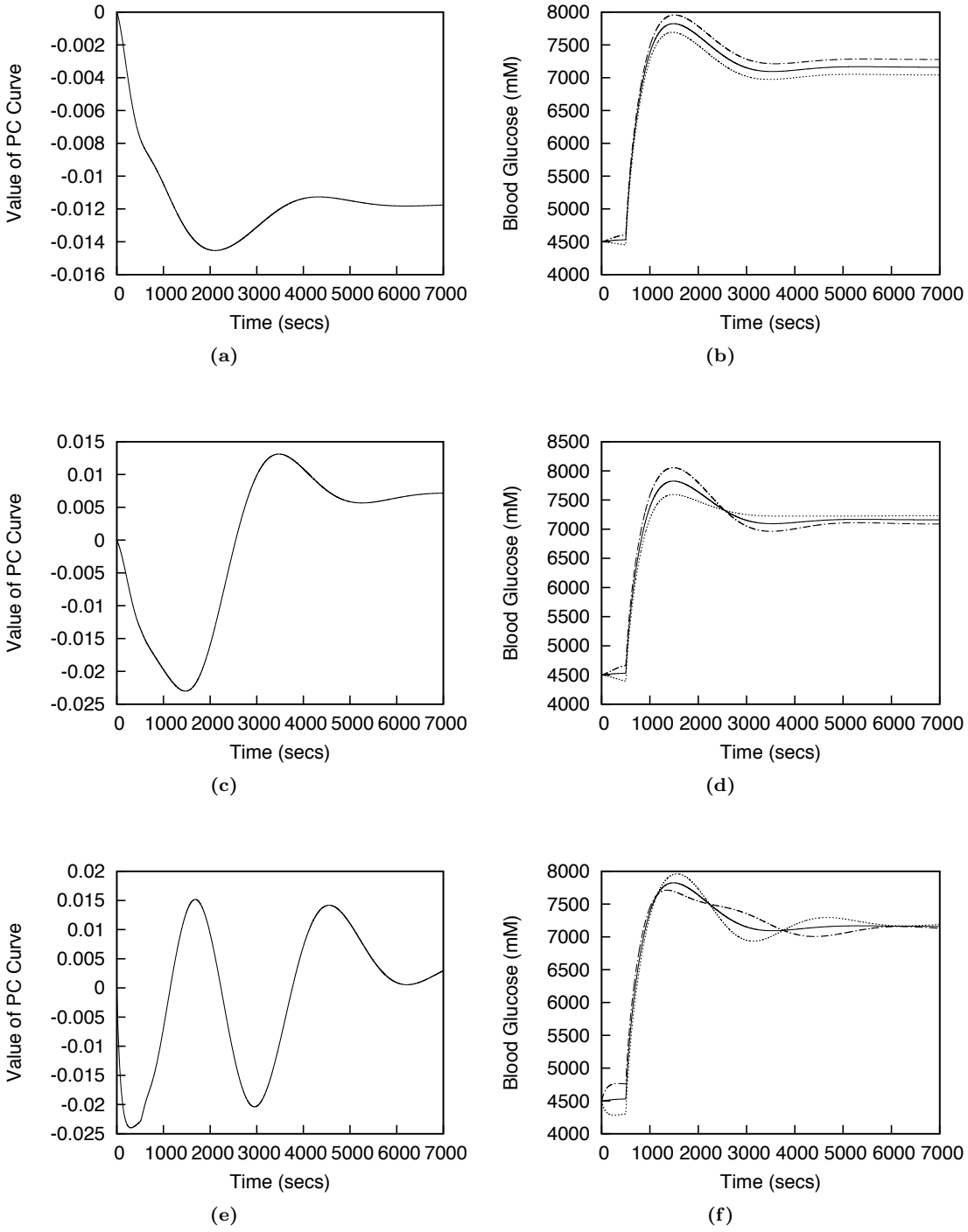


Figure 6.5: The principal components of the composite model output at $M = 15$. The left hand panels show the principal components, the right hand panels show the mean output (solid line) plus (dotted line) and minus (dashed line) a multiple of the corresponding component.

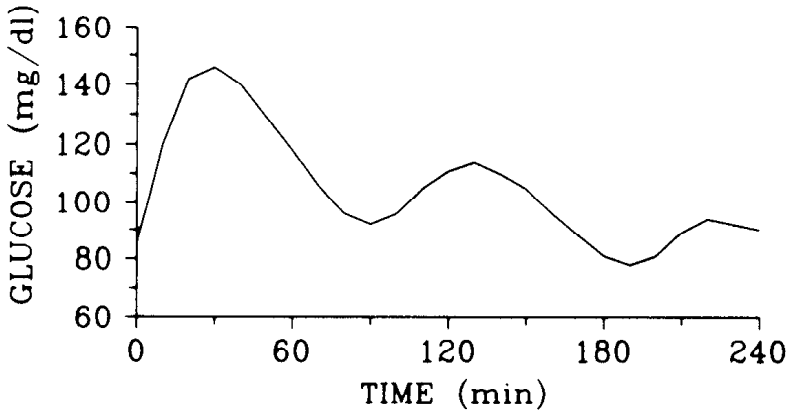


Figure 6.6: Ultradian oscillations in blood glucose observed during oral glucose administration (adapted from Kraegen et al. (1972))

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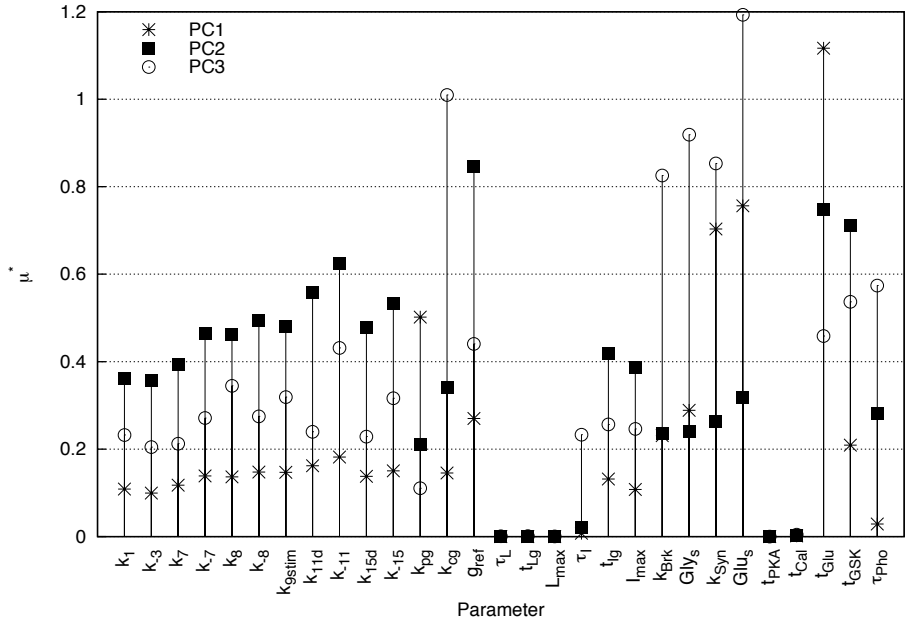
Sensitivities

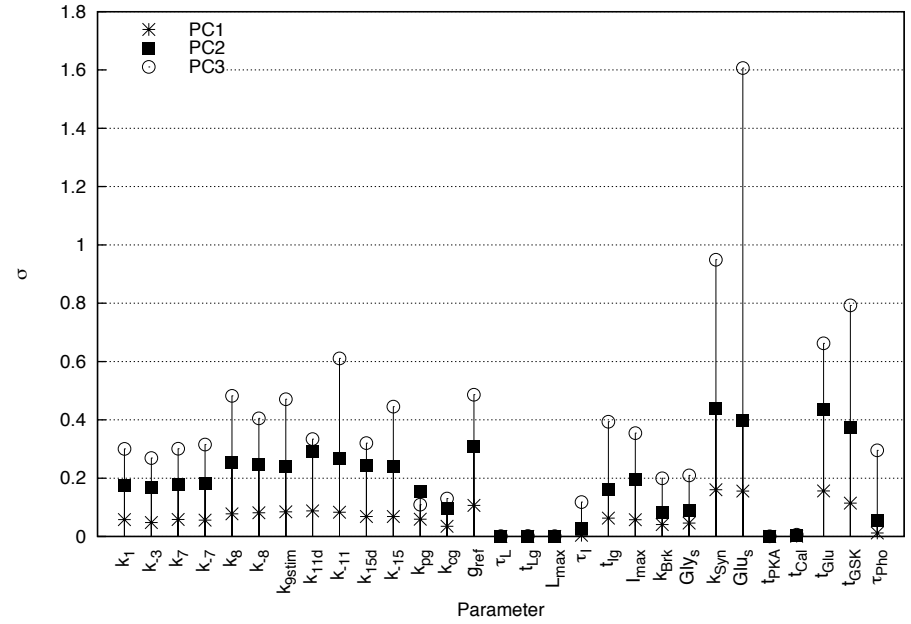
Figure 6.7 shows the sensitivities of the first three principal components to the individual parameters of the composite model. Panel a shows the values of μ^* which provide a measure of the importance of a parameter. Panel b shows the values of σ which indicate the extent to which the importance of a parameter is non-linear or dependent on interactions with other parameters.

The first principal component is primarily controlled by t_{Glu}, k_{Syn} and Glu_s. t_{Glu} is the threshold for blood glucose to regulate hepatic glycogen metabolism suggesting that auto-regulation of glucose production is important in determining the steady state glucose concentration. This is consistent with the view that auto-regulation plays an important role in the maintenance of normoglycemia (Moore et al., 1998). The importance of k_{Syn} and Glu_s is logical as they affect the rate of glycogen synthesis and hence the extent to which hyperglycemia can be avoided by storage of excess glucose as glycogen.

t_{Glu} becomes less important for the other components (PC2 and PC3). This is coupled with an increased importance of the insulin component model parameters (k_1 - k_{-15}) and t_{GSK}, the threshold for GSK3 to affect glycogen metabolism, particularly for PC2. Together these results suggest an increased role of insulin dependent mechanisms in producing the transient excursion in blood glucose and the onset of oscillatory behaviour relative to their effect on the magnitude of the steady state concentration. The importance of the parameters of the insulin model are largely similar. It is therefore difficult to draw any conclusions about the relative importance of specific parts of the pathway on the different types of variation in the blood glucose concentration.



(a)



(b)

Figure 6.7: The principal component sensitivities of the composite model at M=15. Results are shown for the first three principal components. Panel a shows the values of μ^* which measures the total sensitivity. Panel b shows the values of σ which measures the effect of interactions or non-linearities.

The parameters governing glycogen breakdown k_{Brk} and Gly_s are also found to be very important for the occurrence of oscillatory behaviour (PC3). This suggests that the balance between glycogen synthesis and glycogenolysis is important in producing oscillations. This is supported by figure 6.7b which shows that interactions become increasingly important for the higher order principal components.

Interestingly the time-scales of both insulin production in response to glucose (τ_I) and the inactivation of $GPho$ in response to changes in the various signals including inactivation of GSK3 by insulin (τ_{Pho}) are more important in terms of PC3 (which describes oscillatory behaviour) than they are for the other components. Previous studies have concluded that the mechanisms that generate ultradian oscillations are unclear. Two main hypotheses have been proposed: that the oscillations are caused by pulsatile secretion of insulin by the pancreas or that they are the result of the feedback between insulin secretion and glucose production and utilisation (Tolic et al., 2000). A number of models have been developed to investigate the second hypothesis. The results of Sturis et al. (1991) suggest that the occurrence of ultradian oscillations is dependent on the existence of a delay between the production of insulin and its subsequent effects on glucose production. Li et al. (2006) have highlighted the potential role of a second delay, the lag between increases in blood glucose and the secretion of insulin. The hypothesised importance of these time delays is consistent with the results of the sensitivity analysis which show the importance of the corresponding time-scales in the composite model.

6.5.3 Intra and Inter Sensitivities

The previous discussion has focussed on the effects of parameter uncertainties on the system level output. The following section discusses whether any additional information about the system can be uncovered by looking at the component model variables using the ideas of intra and inter sensitivities introduced in chapter 5, section 5.3.

Continuing the focus on the role of the insulin signalling pathway, which motivated the analysis of the principal component based sensitivities presented above, we compare how the parameters of the insulin model affect their own model output (GSK3) and the blood glucose concentration. Figure 6.8 shows the first three principal components of the GSK output together with the μ^* values calculated for the parameters of the insulin component model for an external input of $M = 15$.

The first PC (shown in figure 6.8a) describes a variation in the maximum inactivation of GSK3. PC2 (figure 6.8c) appears to show variation in the time at which GSK3 begins to be inactivated. The third PC shows the possibility of an overshoot in the amount of inactive GSK3 similar to the overshoot in blood glucose shown in figure 6.5d.

The sensitivities show that the same set of parameters are important in all three types of variation. These are the same set of 11 parameters identified as affecting the blood glucose concentration (k_4, k_{-4} and $k_4^{'}$ have a very small effect but these are insignificant compared to the other parameters). An interesting feature is the increased sensitivity to k_{-3} of PC2. This suggests that the rate of deactivation of the insulin receptors may be important in controlling the delay in inactivation of GSK3.

We can compare the sensitivities of the GSK3 output to those of the blood glucose concentration to see whether we can draw any conclusions about the way in which uncertainty in the insulin model effects the system level output. Inspection of figure 6.7 does not highlight any obvious differences in the sensitivities of the glucose output to k_{-3}. This suggest the behaviour described by PC2 is not particularly important in producing variation in the blood glucose output. This idea is supported by the ranking of k_{-11} as the most important of the insulin model parameters with respect to the main modes of variation in the glucose output. This parameter is ranked less important for the second PC of the GSK3 output than for PC1 and PC3.

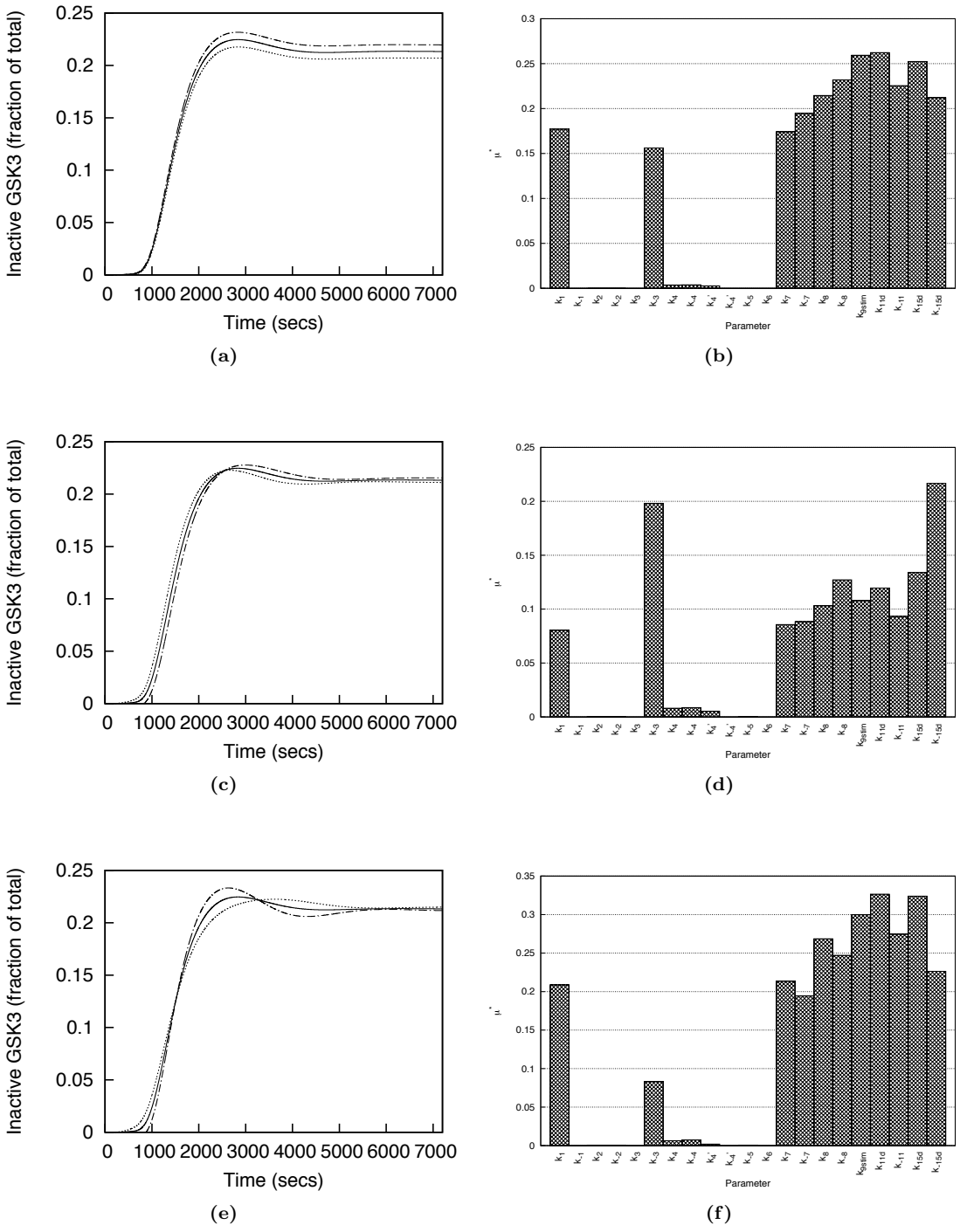


Figure 6.8: The principal component sensitivities of the GSK3 output of the composite model at $M = 15$. The left hand panels show the variation described by the PCs (mean output (solid line) plus (dotted line) and minus (dashed line) a multiple of the principal component). The right hand panels show the sensitivities to the parameters of the insulin component model calculated via the Morris method.

6.6 Discussion

This chapter has presented an application of the methodology for the sensitivity analysis of composite biological models which was developed in chapters 4 and 5. The approaches were applied to the composite model of glucose regulation introduced in chapter 3.

The case study has demonstrated the potential of the approach. The results of the analysis suggest a number of hypotheses about the behaviour of the system and avenues for future model development which are discussed below.

The results of the group level analysis suggest that the role of glucagon dependent calcium activation on blood glucose regulation is of minor importance. The role of calcium in regulating glycogenolysis is still unclear (Aromataris et al., 2006). Our finding is consistent with a number of experimental studies including Pittner and Spitzer (1993) which indicate its role in propagating the glucagon signal is secondary to that of cAMP dependent mechanisms.

Auto-regulation of hepatic glucose production by glucose levels also appears to be important. This is demonstrated by the sensitivity of the model output to t_Glu, the threshold for glucose to influence GPho and GSyn. The importance of auto-regulation in human subjects is well documented (Sacca et al., 1978; Bolli et al., 1985; Rossetti et al., 1993; Moore et al., 1998).

For positive glucose inputs the principal components display two interesting behaviours: a transient "overshoot" in the blood glucose concentration and the possible onset of oscillations in blood glucose (although these are unsustained). It is possible that these oscillations are representative of the ultradian oscillations observed in human subjects during glucose infusion (Kraegen et al., 1972) or enteral nutrition (Simon et al., 1987). The sensitivities of these principal components suggest an increased importance of the insulin model parameters in producing these behaviours. They also suggest that the time-scales of insulin production by the pancreas (τ_I) and changes in the activity of GPho (τ_{Pho}) are important in generating the oscillations. This is consistent with previous modelling studies (Sturis et al., 1991; Li et al., 2006) which hypothesise a crucial role for time delays (between the production of insulin and its subsequent effects on glucose production and increases in blood glucose and the secretion of insulin) in the occurrence of ultradian oscillations.

The results of the analysis can also be used to suggest directions for future developments and refinement of the model. In particular the importance of the glycogenolysis model suggests that this should be the focus of further development. The regulation of GPho and GSyn by the various signals (glucose, cAMP, calcium and GSK3) is currently modelled using fuzzy logic statements. Developing a more mechanistic model of these regulatory processes (which are described in section 3.2.1 and illustrated in figure 3.1) would allow us to investigate their role in the control of blood

glucose levels in greater detail.

The sensitivity analysis also suggests that the glucagon receptor and calcium components could be simplified or possibly removed from the composite model without affecting its ability to describe the regulation of blood glucose levels.

The benefits of the two stage approach, a group analysis followed by an individual parameter analysis were also demonstrated. In particular it was shown that a group analysis can allow us to exclude certain parameters from the more detailed analysis increasing the computational efficiency.

The concepts of intra and inter scale sensitivities proved less successful in the analysis of the composite model. Looking at the sensitivities of GSK3 and glucose to the insulin model parameters did not suggest any obvious additional information about the importance of the parameters of the insulin signalling pathway. This highlights the major limitation of the approach. It is largely exploratory and it may be necessary to look at lots of different combinations of inputs and outputs to identify interesting features of the model. Performing this task via visual inspection of the sensitivity plots is time consuming and difficult. In addition any conclusions could be regarded as subjective. A potential solution to this problem is the development of automated ways to extract information from the sensitivity scores. This idea is explored further in the discussion of future research directions.

Chapter 7

Conclusions

This chapter summarises the work that has been described in this thesis. In section 7.1 conclusions about the use of sensitivity analysis in biological modelling are presented. The contribution of my research is then discussed (section 7.2). Finally some directions for future research are suggested.

7.1 The Use of Sensitivity Analysis in Systems Biology

Biological systems typically consist of large numbers of interacting components and involve processes operating across a variety of spatial, temporal and biological scales. Systems biology aims to understand such systems by integrating information from all functional levels into a single cohesive model. Mathematical and computational modelling is a key part of the systems biology approach providing a method for formally defining and analysing the structure and function of a system.

Sensitivity analysis should be regarded as an important part of the development and use of computational models in systems biology. SA allows us to incorporate parametric uncertainty into the modelling process and to systematically investigate the effects of variations and perturbations of parameter values on the system behaviour.

The results of sensitivity analysis can further our understanding of a system. For example they may provide support to one of a number of competing hypotheses about the important mechanisms underlying the behaviour of a biological system. They may also suggest new hypotheses which can be investigated experimentally.

Sensitivity analysis can also be used to identify control points in a system. This information could be used to suggest potential targets for therapeutic interventions. This presents the future possibility of using computational models and sensitivity analysis techniques for "in silico" drug

identification.

Sensitivity analysis can also be used to drive model development. Attempts to improve the estimation of parameter values should focus on those parameters which are shown to have a significant impact on the model. If the analysis identifies parts of the model which have little effect on the system function it may be possible to remove or simplify these parts to reduce the complexity of the model. Conversely if a particular sub-process significantly influences the model output we should focus on developing or refining that part of the model.

7.2 Contributions

This thesis has attempted to address two main issues with the application of sensitivity analysis in biological modelling: the analysis of time dependent model output and the study of composite or multi-scale models. The contributions of this research to these two problems are discussed in more detail below.

More generally this thesis has investigated the use of global SA techniques in systems biology. Global techniques allow the effects of simultaneous parameter variations across large ranges to be studied. This is important in biological modelling where parameters may vary by significant amounts and interactions between parameters are frequently found to be important. There has been a growing use of global methods in the biological modelling literature in recent years which should be continued. This research has demonstrated the suitability of two SA techniques, the variance based method of Sobol and Morris' screening design. The two methods were found to produce consistent results, the latter at a much lower computational cost.

7.2.1 Analysis of Time Dependent Model Output

When studying biological systems we are often interested in the sensitivity of dynamic model outputs. This thesis has proposed a new approach to the analysis of such systems which is based on a principal component analysis of the model output coupled with a global sensitivity analysis. The approach allows us to investigate the sensitivity of features of the model output rather than the output value at specific time points. These features are extracted directly from the data and are based on the important variation in that data rather than predetermined ideas of what is important. The use of principal components analysis also allows the definition of an overall sensitivity which measures the importance of a parameter on the entire model output.

The approach can be considered as exploratory. If we are interested in a specific type of behaviour of the model there is no guarantee that it will be well captured by the principal components.

In such cases it may be more appropriate to implement a computational algorithm to extract a scalar measure of the feature of interest directly from the data and apply sensitivity analysis to this value.

7.2.2 Analysis of Composite Models

The second focus of my research has been the analysis of composite models which describe biological systems consisting of processes at different scales or in different components. This thesis has suggested two complimentary approaches to the analysis of such models.

The use of group sensitivity analysis has been demonstrated as a way to investigate the importance of the different components of the system. This approach, which utilised a modified version of the Morris method was shown to produce useful results. In addition it provides a way to reduce the computational cost of a full analysis by identifying non-influential parameters which could be excluded from future SA.

The potential of intra and inter scale sensitivity analysis was also investigated. This approach suggests applying SA both within (intra) and across (inter) components to identify interesting patterns in the sensitivity of the model.

7.2.3 Analysis of Glucose Homeostasis Model

The methodology has been applied to a composite model of blood glucose homeostasis. This case study demonstrated the potential of the approach on a real system of interest. The results suggest a number of interesting points about the system:

- The mobilisation of intracellular calcium in a glucagon dependent manner plays a minor role in the regulation of glycogen metabolism
- Auto-regulation of hepatic glucose production by glucose is important in regulating blood glucose levels
- Time delays between changes in blood glucose, the production of insulin by the pancreas and the effect of the hormone on hepatic glucose production are important in the possible onset of ultradian oscillations

7.3 Directions for Future Research

7.3.1 Analysis and Development of the Glucose Homeostasis Model

An immediate extension of the research presented in this thesis is to use the proposed methodology to explore the composite blood glucose model in greater detail. In particular it would be of interest to study the sensitivity of the model using parameter ranges representative of different disease states, specifically diabetes. This may provide information on the mechanisms which are most important in producing the system behaviours which are observed in patients with diabetes or other conditions.

The results of the analysis presented in chapter 6 could also be used to direct development of the model. In particular the SA results suggest that the glycogenolysis model is important in producing the observed variation in the model output. This process is presently modelled using a fuzzy logic approach in which the effects of the various regulatory signals are modelled as simple threshold functions. The development of a more mechanistic model of the regulation of glycogen phosphorylase and glycogen synthase would allow us to investigate the role of these processes in the control of blood glucose in greater detail.

7.3.2 Automation of the Processing of SA Results

Sensitivity analysis of biological models can produce large amounts of sensitivity data which needs to be processed. The amount of data is increased by the use of the methods presented in this thesis which suggests looking at multiple model outputs across multiple scales or components.

The results of a sensitivity analysis are typically processed via visual inspection of the sensitivity measures in tabulated or graphical form. It would be interesting to investigate the use of automated procedures for processing the SA results, for example by defining threshold values for importance or insignificance against which the sensitivity measures could be compared. Automated approaches may be particularly useful in identifying interesting features in an intra and inter scale analysis which may not be apparent from visual inspection alone.

7.3.3 Improvement of Standard SA Techniques

The work in this thesis has made use of standard SA techniques, combining them with other procedures (e.g. PCA) to develop a methodology for sensitivity analysis of biological models. There is potential for developing new standard methods to be used in this framework.

Of the methods utilised here the Morris method provides an efficient way to identify important

and non-influential parameters in a model. The cost of this efficiency is a lack of a rigourous quantification of the contribution of a parameter to the output uncertainty. The variance based methods, including the method of Sobol, provide such a measure by calculating the reduction in the variance of the model output which could be achieved by fixing a given model parameter. However such methods have a high computational cost which makes them unsuitable for the analysis of systems containing large numbers of parameters.

The qualitative ranking provided by the Morris method is suitable for exploratory style analysis. However future applications to biological systems may require a more quantifiable measure of sensitivity. If the aim is to design interventions for regulating the system output it would be important to quantify the effects of perturbing different targets. Therefore a goal of further research is the development of SA methods which combine the computational efficiency of the Morris method with the sort of quantitative measures provided by the method of Sobol.

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List of Abbreviations

Akt	Akt Protein Kinase (Also known as protein kinase B (PKB))
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
cAMP	Cyclic Adenosine Monophosphate
CICR	Calcium Induced Calcium Release
CDF	Cumulative Distribution Function
DDM	Decoupled Direct Method
eFAST	Extended Fourier Amplitude Sensitivity Test
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Endoplasmic Reticulum
ERK	Extracellular Signal-Regulated Kinase
FAST	Fourier Amplitude Sensitivity Test
GDP	Guanosine Diphosphate
Glc-6-P	Glucose-6-Phosphate
GPCR	G-Protein Coupled Receptor
GPho	Glycogen Phosphorylase
GSyn	Glycogen Synthase
GSK3	Glycogen Synthase kinase
GTP	Guanosine Triphosphate
IFFD	Iterated Fractional Factorial Design
INSR	Insulin Receptor
IP3	Inositol Trisphosphate
IP3R	Inositol Triphosphate Receptor
IRS	Insulin Receptor Substrate
JAK	Janus Kinase
LHS	Latin Hypercube Sampling
MAPK	Mitogen-Activated Protein Kinase
MCA	Metabolic Control Analysis
MC	Monte-Carlo
NF-κB	Nuclear Factor κB
OAT	One-at-a-time
ODE	Ordinary Differential Equation
PBPK	Physiologically-Based Pharmacokinetic
PC	Principal Component
PCA	Principal Component Analysis
PDF	Probability Density Function
PDK1	Phosphoinositide-Dependent Kinase 1
PI3K	Phosphoinositide 3-Kinase
PIP3	Phosphatidylinositol (3,4,5)-Trisphosphate
PKA	cAMP-Dependent Protein Kinase
PKB	Protein kinase B
PLC	Phospholipase C
PRCC	Partial Rank Correlation Coefficient
PTP	Protein Tyrosine Phosphatase

RSA	Regionalised Sensitivity Analysis
RTK	Receptor Tyrosine Kinase
SA	Sensitivity Analysis
SB	Sequential Bifurcation
SERCA	Sarco/Endoplasmic Reticulum Calcium ATPase
STAT	Signal Transducers and Activators of Transcription
VCE	Variance of the Conditional Expectation

Nomenclature

B^*	Morris method input matrix
d_i	Elementary effect of the i^{th} parameter
E	Expected value
G	Number of groups in the group Morris method
G^*	Group perturbation matrix for the Morris method
N	Number of model evaluations
p	Number of levels in the Morris method
q	Maximum number of principal components
q_s	Subset of PCs used in overall sensitivity measures
r	Number of sample points or elementary effects for each parameter in the Morris method
S_i^j	First order Sobol index for j^{th} output for i^{th} parameter
S_{Ti}^j	Total effect Sobol index for j^{th} output for i^{th} parameter
S_i^O	Overall first order Sobol index for i^{th} parameter
S_{Ti}^O	Overall total effect Sobol index for i^{th} parameter
S_i^{Avg}	Time averaged first order Sobol index for i^{th} parameter
S_{Ti}^{Avg}	Time averaged total effect Sobol index for i^{th} parameter
T	Total number of time-points in model solution
V	Variance
V_{PC}^z	Variance described by the z^{th} principal component
$\mathbf{X}$	Vector of model parameters
x_i	i^{th} element of $\mathbf{X}$
y_i	Output of the i^{th} model evaluation
z	Index of PC
Δ	Step size in the Morris method
$\hat{\mu}$	Mean model output
$\bar{\mu}$	Time average of $\hat{\mu}$
μ_i	Mean of the elementary effects of the i^{th} parameter
μ_i^*	Mean of the absolute elementary effects of the i^{th} parameter
μ_O^*	Overall Morris measure for i^{th} parameter
σ_i	Standard deviation of the elementary effects of the i^{th} parameter
Φ	Set of basis functions
ϕ_k	The k^{th} basis function in Φ
Ω_k	Set of coefficients of the k^{th} basis function
ω_{ik}	Coefficient of the k^{th} basis function for the i^{th} model evaluation