Hierarchical modeling of diabetes – a pilot study

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LiU-IKE-EX—2009/14
Final Thesis

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Abstract

In type 2 diabetes the concentration of glucose in the blood is increased, and tissues like fat and muscle become less sensitive to insulin. These two phenomena are interrelated, but since the glucose-insulin interplay is highly complex, many aspects are still not understood. Here, a model-based approach might help. Nevertheless, also a model-based approach has a limited impact, unless models for the sub-systems can be combined into a model for the whole-body regulation. Such a multi-level, module-based model is referred to as a hierarchical model, and this thesis is a proof-of-principle study for the future development of such models.

We have extended one of the best available models for the whole-body regulations, to include a zoomable module for the fat tissue. The first step was to implement the whole-body model in the software MathModelica, which support hierarchical modeling. Second, the originally merged insulin-responding module was sub-divided, so that a fat tissue was singled out. Third, a model for the input-output profile for the fat tissue was developed by combining mechanistic knowledge with existing and novel data from human fat cells. Finally, this detailed model was fitted to the profile of the original fat model, and inserted in the whole-body model, with negligible effect on the whole-body simulations.

The resulting model has the ability to translate mechanistically oriented simulations on the biochemical level, which is the level were drugs act, to the whole-body level, which is of clinical interest. This is a quantum leap forward for modeling, and understanding, glucose homeostasis and type 2 diabetes.
Acknowledgments

I would like to thank…

…Peter Strålfors, my examiner, for the time you spent discussing this project with me and for giving me valuable inputs.

…Gunnar Cedersund and Jan Brugård, my supervisors, for your support and help during this project.

…Claudio Cobelli, Chiara Dalla Man, and Morten Gram Pedersen for providing me valuable insights and simulation files, both regarding the whole-body model, and regarding possible future extensions on beta cell models.

…Cecilia Johansson, my roommate at Cell biology, for your interest in my project and the time you have spent discussing any questions I have had.

…Karin Gustafsson, my roommate at MathCore, for taking extra care of me the first time and for all nice small talks in our room.

…Anita Öst, my roommate at Cell biology, for extra glucose uptake experiments and advises in how to calculate the uptake from radiation data.

…All of you friendly persons at MathCore and Cell biology for taking good care of me.

…Tomas Lindblom, Ann Winzell and Erik Nilebäck, my friends and classmates during my education, for all the time we spent together at NH deep down in the course literature.

…Anders Hedlund, for giving me valuable comments on my report and for always being by my side.

This master thesis was made possible thanks to the BioBridge project, www.BioBridge.eu. The BioBridge project is supported by the European Commission and is part of the Sixth Framework Programme.
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1 Introduction

Type 2 diabetes is a common and widely studied disease. It is characterized by high levels of glucose in the blood, and by insulin resistance in the target organs. The interplay between glucose and insulin is highly complex, which makes a model-based approach preferable. In this chapter reviews will be made of the glucose-insulin interplay, both on the whole-body and on the cellular level. We will also have a look at some of the existing models for the whole-body level. In the end of the chapter the objectives of the thesis are stated: to hierarchically extend an existing mathematical model for the whole-body level, for the purpose of including a zoomable module for the adipose tissue.
1.1 Problem formulation

To achieve a detailed and consistent understanding of a physiological system in the human body, many research teams around the world have to be involved. This is the case since research teams typically work with partial systems or aspects, such as organs, hormonal effects or the clinical whole-body level. The knowledge of these partial systems must be combined. For such a combination, one approach is to use so called hierarchical models with a multi-level structure (Figure 1.1). In this thesis, one level corresponds to e.g., the whole-body, and another to the organs. The top-level would then deal with the flows between the organs and the organ level would describe the details within the organs. Importantly, the hierarchical structure makes it possible to replace the sub-models of the partial systems, when more knowledge is available – without affecting the rest of the model.

Figure 1.1.
A hierarchical model is a multi-level model with a tree-structure. The sub-models can easily be interchanged. The picture shows two examples of hierarchical model structures.

The start in this project is an existing whole-body model by Dalla Man et al. [2007] describing the glucose and insulin concentrations and flows after a meal. The task is to make the model hierarchical using knowledge from the research team Peter Strålfors laboratory at the Department of Experimental and Clinic Research at Linköping
University. An adipose tissue module describing the glucose uptake by adipocytes should be developed and integrated as an underlying level in the whole-body model.

A detailed model of the glucose-insulin system can in the extension be used when studying different factors that affect diabetes and give a further understanding in how to treat patients.

1.2 Whole-body aspects of type 2 diabetes

Type 2 diabetes is one of the most common diseases worldwide. The World Health Organization [2008] estimates that over 180 million people are suffering from it, and the number is continuously increasing. Unlike type 1 diabetes, the patients’ pancreas can usually produce insulin at normal levels; the problem is instead the ineffective use of the insulin produced. The adipocytes and muscle cells become less and less sensitive to insulin, which leads to increased levels of glucose in the bloodstream. Over time, diabetes can damage the heart, blood vessels, eyes, kidneys and nerves [The World Health Organization 2008]. Both genetics and environmental factors – such as excess body weight and physical inactivity – influence the outbreak of type 2 diabetes. Thus, the first step in treatment of the disease is a change of diet and more physical activity [LeRoith et al. 2003].

1.3 Cellular aspects of type 2 diabetes

Also on a cellular level in type 2 diabetes there are many aspects to consider. One of these aspects is the insulin signaling network. Many research teams attempt to understand the mechanisms in the network and more details are discovered all the time. In this
project the main interest is the path of the network that controls glucose transport in the adipocytes and muscle cells. As described by LeRoith et al. [2003], the process starts with insulin molecules that bind to the insulin receptor (IR) in the cell membrane. This activates (phosphorylates) IR. Active IR phosphorylates the insulin receptor substrate protein 1 (IRS1) inside the cell on selective tyrosine sites. These phosphorylations are used as docking sites by downstream effector molecules. The next important step in this path is the activation of protein kinase B (PKB). PKB regulates the translocation of vesicles including glucose transporter 4 (GLUT4) from the cytosol to the plasma membrane. The vesicles merge into the membrane where GLUT4 starts to transport glucose from the interstitial fluid to the inside of the cell. This signaling cascade is shown in Figure 1.2.

**Figure 1.2.**
A simplified drawing of the insulin signaling pathway from insulin attachment to insulin receptor (IR) that activates insulin receptor substrate protein 1 (IRS1) and protein kinase B (PKB) and finally vesicles holding glucose transporter 4 (GLUT4). The vesicle moves to the plasma membrane and GLUT4 transports glucose into the cell. Glucose transporter 1 (GLUT1) continuously transports glucose into the cell for the basal needs.
Another important protein, involved in glucose homeostasis in adipocytes, is glucose transporter 1 (GLUT1). While the task of GLUT4 is to rapidly lower the glucose concentration in the blood plasma after a meal, GLUT1 continuously delivers glucose required for basal cellular activity. In most human cells GLUT1 primarily exists in a combination with tissue specific glucose transporters [Mueckler 1994].

The malfunctions in the insulin signaling network in the cells of type 2 diabetics can be of different kinds. Mutations in the gene expressing IR do exist but are rare. Phosphorylation of IRS1 can be both increased and decreased. Increased IRS1 phosphorylations function as a steric hinder for downstream signaling. There can be defects at numerous points in the regulation system of the glucose transport, but the GLUT4 glucose transporter is expressed at normal levels in type 2 diabetes [McCarthy and Elmendorf 2007]. All in all, there are still crucial connections to be understood between the insulin signaling pathway and type 2 diabetes.

1.4 Modeling tools

The tools used in this project are the computer programs MathModelica and MATLAB. MathModelica System Designer is a modeling program for analysis of dynamic systems, mainly used in the field of mechanics, but also in systems biology. The program language used is Modelica, which is the one of the most used object-orientated modeling language today. MathModelica has a graphical environment and is built up by component libraries for different usage [MathCore Engineering AB 2009]. The BioChem library is used for biological applications [BioChem 2008]. MathModelica support Systems Biology Markup Language (SBML), which is used to translate biological models between different programs [MathCore Engineering AB 2009].
MATLAB is a widely used technical computing language tool. The most common applications are algorithm development, data visualization, data analysis, and numeric computation. Toolboxes in MATLAB are collections of functions of a special purpose [The MathWorks 2009]. One such toolbox is the systems biology toolbox for MATLAB (SBTB), which includes tools for creating models of biological systems [Schmidt and Jirstrand 2005]. A model created can be represented in two ways. One way is as differential equations and the other way is as biochemical reactions. The toolbox can handle Systems Biology Markup Language (SBML).

1.5 Existing models of the glucose-insulin system

There are some existing models describing the glucose-insulin system in the human body. They use a variety of approaches and formulate the models at different degrees of detail. The most common approach is probably pharmacokinetic and pharmacodynamic models (PKPD). Such models are typically simple with only a few states [Cedersund and Strålfors 2009]. The models may often suffer from unrealistic parameter values and/or states which lack a clear biological interpretation. There are on the other hand also a few models which are more physiologically realistic. Since, in this thesis, the attempt is to formulate a hierarchical model, the latter models with realistic details are of most interest. Here follows a review of three of the most important such models. The first of these is the chosen model for further development into a multi-level hierarchical model.

1.5.1 Dalla Man model

The Dalla Man model is a glucose-insulin model of flows and concentrations of the substances during and after a mixed meal. It is described in an article by Dalla Man et al. [2007]. The main reason for the Dalla Man model to be created was an access to
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unusually informative data of insulin (I) and glucose (G) concentrations in the plasma. Measurements had been done during 420 minutes during and after an intake of a mixed meal and as many as 204 persons were included in the study. The same measurements had also been performed in 14 persons with type 2 diabetes. The model explained normal data with one parameter set and data from diabetics with another parameter set.

Figure 1.3. A sketch of the Dalla Man model. The white circles represent the states in the model. The blue arrows show the glucose flows and the pink arrows show the insulin flows. Thin, dotted arrows indicate an information exchange between the subsystems.
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The meal was labeled with radioactivity to measure the glucose flow through the gastrointestinal tract. To be able to estimate more flows in the body, two additional labeled tracers were infused intravenously and estimations were made by using the a complex tracer-to-tracee ratio clamp technique as described in Basu et al. [2003].

The following flows were estimated with the technique:

- Rate of appearance (Ra) = glucose flow from the intestine to plasma
- Endogenous glucose production (EGP) = glucose flow from liver to plasma
- Utilization (U) = glucose flow from plasma to tissues
- Secretion (S) = insulin flow from beta cells to the liver

The reason for these estimations was previous knowledge that measurements of G and I alone were not enough to validate the model: many different EGP, Ra, S and U curves can give the same G and I curves. The subsystems in the Dalla Man model are connected with the concentration and flux of insulin and glucose. A graphical view of the model can be found in Figure 1.3 and all model equations plus parameter values, are found in appendix 1.

The adipose and muscle tissues in the Dalla Man model account for the insulin dependent glucose uptake \( (U_{id}) \) of all the body. The uptake is described by a Michaelis-Menten expression:

\[
U_{id}(t) = \frac{V_m(X(t)) \cdot Glu_{tissues}(t)}{K_m + Glu_{tissues}(t)}
\]

where \( V_m \) is a function of insulin near the cells (denoted X) and describes the maximal uptake. The more insulin, the more GLUT4 in the cell membrane and the more glucose is transported into the cell. \( K_m \) is the Michaelis-Menten constant. The utilization is saturated
in glucose around the cells \( (\text{GLU}_{\text{tissues}}) \). That is, the utilization will not be faster than \( V_m \) no matter the glucose concentration around the cells.

Except for the glucose uptake by muscle and adipose tissue, some other organs are also involved: the gastrointestinal tract, the liver, and the pancreas. The liver is sensitive to insulin levels in plasma and in the portal vein (insulin secreted from beta cells), as well as glucose levels in the plasma. According to these levels the liver produces more or less glucose. The pancreas consists of insulin producing beta cells. These cells respond to high glucose levels in the plasma. The brain and the red blood cells utilize a lot of glucose non-dependently of insulin. All insulin-independent glucose uptakes are gathered in one constant glucose uptake \( (U_{ii}) \). Glucose excretion to the kidneys is also included in the model. This excretion only occurs when glucose values passes a threshold levels – a symptom of diabetes. The model of the gastrointestinal tract has been described and tested in another article by Dalla Man \textit{et al.} [2006], “A system model of oral glucose absorption: validation on gold standard data”. That model describes the glucose absorption by the intestine after an oral intake.

In this project, the Dalla Man model will be developed in three steps. First the model will be made hierarchical by using the program MathModelica. Then the tissues module will be divided into two different modules; one describing the muscle tissue and one the adipose tissue. In the third step a more detailed model of the adipose tissue, based on knowledge and data on the cellular level, will be developed, and included in the then hierarchically extended multi-level version of the model.

### 1.5.2 Model of fuel homeostasis during exercise

In the article “Multi-Scale computational model of fuel homeostasis during exercise: Effect of hormonal control” by Kim \textit{et al.} [2007] one can find another existing model where glucose and insulin plays a major role. The model describes what takes place in the
human body during exercise. Not only is the hormone insulin included in the model, but also glucagon that stimulates the conversion of glucose to glycogen – the reverse effect of insulin. The included substances range from glucose and glucose products like glucose-6-phosphate and glycogen to fatty acids, ATP/ADP, CO₂ and O₂. Muscle and adipose tissue are in this model divided and modeled one by one. This is a complex and detailed model but, at least compared to the Dalla Man model, it is based on poor raw data.

### 1.5.3 Glucose homeostasis model

Normann Hansen [2004] has studied modeling of glucose homeostasis in health and diabetes. The studies are summarized in the PhD thesis “Glucose homeostasis: A biosimulation approach”. The standard model in that thesis is a model of the glucose-insulin system with the three states plasma insulin, plasma glucose and hepatic glycogen. A drawing of the model can be found in Figure 1.4.

![Figure 1.4](image)

*Figure 1.4.*

*A view of the glucose homeostasis model by Normann Hansen R [2004]. The circles represent states and thick arrows flows. Thin, dotted arrows represent control of the flows.*
The uptake of glucose in tissues is modeled the same for muscle and adipose tissue, with one insulin (I) dependent and one glucose (G) dependent part.

\[ f(G, I) = \frac{a \cdot G}{K_g + G} \cdot \frac{J \cdot I^\alpha}{K_i^\alpha + I^\alpha} \]

\( K_g, K_i, a \) and J are constants and \( \alpha \) is the Hill coefficient.

### 1.6 Purpose

- To use the modeling tool *MathModelica* to translate the Dalla Man model into a hierarchical form, i.e. with an ability to zoom in the different organs.
- To divide the tissue module in the existing model into two parts, describing the muscle and adipose tissue, respectively. The division should be based on studies of glucose uptake in these tissues.
- To create and insert a detailed, mechanistic adipose tissue module into the whole-body model. The model should be based on knowledge of insulin signaling and glucose uptake in adipocytes.

A whole-body model of the glucose-insulin system with mechanistic details on a cellular level can be used to study the effect of intra-cellular interactions on the whole-body level, which is the pharmaceutically interesting level. In the extension, diabetes research can benefit enormously from such models. Another purpose of making a computational model is that it can be used to simulate actions, drugs and other course of events, and thus reduce the use of laboratory animals.
1.7 Delimitations

- In the model only the glucose utilization module will be improved. Knowledge of the biology behind the other modules will not be used.
- The glucose and insulin concentrations and flows are the only metabolites and hormones that are included in the original Dalla Man model: these factors will not be improved within this project.
- The time of the project is 20 weeks.
In this project a novel approach within the field of systems biology have been used: hierarchical modeling. This hierarchical modeling approach has been inspired from fields like mechanics and electrics, where such models have been used for a long time. A hierarchical model of a biological system like the glucose-insulin system can be used to study whole-body effects of changes on a cellular level. These kinds of studies could in the long run lead to a more consistent and coherent understanding of type 2 diabetes.
2.1 Modeling of biological systems

When creating models of biological systems, both experimental data and knowledge of the system (or at least a hypothesis) is required [Tomlin and Axelrod 2007]. Models of biological systems, as well as other dynamical systems, are often composed by a set of ordinary differential equations. These equations model how the variables changes with time [Reeves and Fraser 2009]. A simple biochemical reaction and the corresponding differential equations can be found in Figure 2.1. There are also other ways to model biological systems, e.g. Bayesian networks, Boolean networks, and stochastic equations [de Jong 2002].

\[
\begin{align*}
\frac{d}{dt}(S_1) &= k_b \cdot S_2 - k_f \cdot S_1 \\
\frac{d}{dt}(S_2) &= k_f \cdot S_1 - k_b \cdot S_2
\end{align*}
\]

**Figure 2.1.**
A) Two substances, $S_1$ and $S_2$, in a biochemical reaction. The rate parameters, $k_f$ and $k_b$, describe the flow between the substances. B) The differential equations communicate the changes in $S_1$ and $S_2$ with time.

The kinetics of biological systems is often non-linear in a way that can be described using Michaelis-Menten kinetics. More information is given in the box below.
Michaelis-Menten kinetics

The simple Michaelis-Menten expression describes the kinetics of an irreversible reaction catalyzed by an enzyme. The Michaelis-Menten equation relates the initial reaction rate, $V_0$, to the substrate concentration, $[S]$:

$$V_0 = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]}$$

The resulting graph is a hyperbolic function;

where $V_{\text{max}}$ is the maximal reaction rate, and where the Michaelis-Menten constant $K_m$ corresponds to half of the maximal reaction rate ($V_{\text{max}}/2$).
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The creation of mathematical models of biological system can be separated in two main approaches: bottom-up and top-down modeling. In the bottom-up approach one starts with models of mechanistic details, and combines those step by step into larger and larger models. The idea is to start with an understanding of the details inside the components and their interactions, and later on understand the functions of the whole system. Bottom-up modeling requires measurements and knowledge on a mechanistic level. Top-down modeling works the other way around; one starts with a simple functional model of a high level system, based on experimental data of that system. This top-level model can then be improved and made more realistic with more detailed knowledge [Cedersund and Strålfors 2009, Tomlin and Axelrod 2007]. The merging of the two mentioned modeling approaches is called hierarchical modeling [Cedersund and Strålfors 2009]. In this project a hierarchical modeling approach is applied.

There are many difficulties in the modeling of biological systems. First, it is not possible to measure all interesting variables. Second, there are experimental differences both between samples and within the same sample, and finally; the studied systems are nonlinear and complex. Nevertheless, (or perhaps because of these reasons), modeling in this field is highly necessary since new experimental techniques produce increasing number of data that are impossible to evaluate without computer power [Mogilner et al. 2006].

2.2 Hierarchical modeling and MathModelica

A hierarchical model is a model in several levels with a tree-structure, see Figure 1.1. The benefits with a hierarchical model formulation are that components can be reused, and that the modeler is forced to connect the modules in a way that makes it easy to replace them without an effect on the rest of the model. The main advantage of using hierarchical modeling when modeling biological systems is that is becomes possible to simulate high-
level effects of changes on a low level. For example changes on a protein level can be simulated to see the whole-body effect. Hunter and Borg [2003] describe the acknowledged Physiome project; a project for merging sub-models on different levels from protein level to the whole organism. When combining such models, there are huge differences in spatial- and time-scales, and a way to overcome such problems is by using hierarchical modeling. So far, however, the Physiome project has mainly dealt with the heart, and very little has yet been done for the study of diabetes.

A one-level model is on the other hand referred to as a flat model. In such a model there is no reuse of structures and it is generally harder to replace parts of the model.

Modelica is an object-oriented language suitable for large and complex modeling. The language support multi-domain modeling, i.e. different fields, like electrics, mechanics and hydraulics, can be included in the same model. Modelica can handle differential, algebraic and discrete equations [Otter and Elmqvist 2001].

Other examples of modeling languages used for modeling of biological systems are SBML [The Systems Biology Markup Language 2009] and CellML [CellML 2009]. These languages can only be used for flat, non-hierarchical models.

Dymola and MathModelica are two examples of modeling-softwares that support the language Modelica. The latter was used in this project and is now described further. MathModelica is a program that is mostly used for mechanical and electrical applications, but also, since a few years, for some biochemical applications. The program supports hierarchical modeling because of the object-oriented language Modelica. An important feature in order to build reusable models in MathModelica is to define partial models. The partial models can then be included in new created models to inherit the functions of the partial model. More information about MathModelica can be found at MathCore Engineering AB [2009].
When a model of a biological system is created in the program *MathModelica*, the BioChem library is used. The library is described in the article Larsdotter Nilsson and Fritzson [2003]. The main components of the library are substances and reactions. The substances represent states or variables. One kind of substance included in the project act as buffers because their contents do not change during simulations. Figure 2.2 shows the symbols of substances and reactions in BioChem library.

Reactions in the BioChem library describe the flow between the states and variables. The reactions can be activated, modified or inhibited by other substances. It is possible to create unique reactions to the applications required, but there are some rules that must be followed [MathCore Engineering AB 2009].

The very first step in the project was to translate the model by Dalla Man et al. [2007] to a hierarchical model by using *MathModelica*. All equations from the article were translated to substances and reactions in the BioChem library and, when needed, new reactions were created. A model with a flat, one-level structure was also created to validate the simulations of the hierarchical one. A modeling example in *MathModelica* can be found in the following box.
Example of modeling in MathModelica

Consider the following system and differential equations:

\[
\frac{d}{dt}(S1) = kb \cdot S2 - kf \cdot S1 \cdot M1
\]

\[
\frac{d}{dt}(S2) = kf \cdot S1 \cdot M1 - kb \cdot S2
\]

Let us now see how this system is formulated in MathModelica. The diagram view in MathModelica shows the substances and the activator as circles and the reaction as an arrow. These objects are connected by the user to achieve information transfer. The objects are available in the BioChem library. Apart from the diagram view, there is also a text view available in MathModelica. The views for the simple model above are as follows:

**DIAGRAM VIEW**

**TEXT VIEW**

```model Example
BioChem.Reactions.Activation.Uar reaction;
BioChem.Substances.Substance S1;
BioChem.Substances.Substance S2;
BioChem.Substances.SignalSubstance M1;
equation
connect(M1.n1, reaction.a1);
connect(reaction.p1, S2.n1);
connect(S1.n1, reaction.s1);
end Example;
```
2.3 Calculations

In glucose uptake experiments performed by Peter Strålfors laboratory, adipocytes were stimulated with insulin in different concentrations for 30 min. Then, 2-deoxy-D-glucose mixed with radio labeled glucose (2-deoxy-D-[H\textsubscript{3}]-glucose) was added to the solution and the radioactive decay was measured after incubation. This decay is proportional to the glucose absorbed by the adipocytes. An example of calculations of the glucose uptake rate from experimental data follows below.

Example of calculations after experimental glucose uptake in adipocytes

\[
\begin{align*}
\text{Glucose mixture:} & \quad \begin{cases} 
7.5\mu\text{L of 45 mM 2-deoxy-D-glucose} \\
7.5\mu\text{L of 0.11 mM 2-deoxy-D-[H\textsubscript{3}]-glucose} \\
750\mu\text{L KRH0G}
\end{cases}
\end{align*}
\]

25 \mu\text{L} of the glucose mixture, 10 \mu\text{L} insulin in different concentrations and 190 \mu\text{L} 10% adipocytes were added to each test tube.

\[
\begin{align*}
7.5\mu\text{L} \cdot 45\text{ mM} & = 0.3375 \mu\text{mol 2-deoxy-D-glucose} \\
7.5\mu\text{L} \cdot 0.11\text{ mM} & = 0.825 \mu\text{mol 2-deoxy-D-[H\textsubscript{3}]-glucose} \\
0.825 / (337.5+0.8325) & = 0.24 \% \text{ of the glucose molecules were labeled}
\end{align*}
\]

In one test tube there is \(25 / (750+7.5+7.5) = 3.27 \%\) of the glucose mix  
=> \(0.0110 \mu\text{mol 2-deoxy-D-glucose}\)  
=> \(0.0198 \text{ nmol 2-deoxy-D-[H\textsubscript{3}]-glucose}\)

Measurement (without adipocytes): 180000 decays/min = 3000 decays/s = 81 nCi
81 nCi corresponds to 0.011 µmol total glucose

=> 7360 Ci/mmol glucose

Measurement (glucose uptake): 4000 decays/min = 66.67 decays/s = 1.80 nCi

1.80 nCi corresponds to 1.80 nCi / 7360 Ci/mmol = 0.24 nmol

0.24 nmol glucose per 30 min per 19 µL adipocytes:
=> 420 nmol/min per L cells
=> 8.8 µmol/min per human body á 21 L adipose tissue
=> 1.6 mg/min per human body
=> **0.02 mg/min per kg** (78 kg person)

Glucose concentration: 0.011 µmol / (190+10+25) µL = 0.049 mM

### 2.4 Optimization

The optimization of the parameters in the model describing an adipocyte was made in the systems biology toolbox for MATLAB. Functions in MATLAB were created to simulate the model and to calculate a cost depending on the distance between the simulated and the experimental data. The equation calculating the distance, i.e. the cost, has the following appearance:
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\[ V(p) = \sum \left( \frac{y - \hat{y}}{r} \right)^2 + \text{punishment} \]

where \( V(p) \) is the distance, \( y \) the experimental data, \( \hat{y} \) the simulated value and \( r \) is a normalizing parameter [Cedersund 2006].

To help the functions find fitting parameters, if-statements were inserted into the cost-function with things to be “punished”. For example a simulation can be punished if the highest point is too low or is located at an incorrect time according to experimental data. The reason for these modifications is to help the functions to find its way around in the parameter space. This is sometimes needed when a high number of parameters are optimized. The parameters that receive a low cost in a simulation are saved and compared when the optimization is ready.

The optimization method used in this project is a combination of the global simulated annealing approach with the downhill simplex method, which can be studied in Press et al. [1992]. The task of the method is to find the minimum of a function with more than one independent variable. There exist other optimization methods for multi-dimensional modeling as well [Cedersund 2006].

It should be noted that in this project, the optimization data came both from simulations of the top-level whole-body model and from measurements on extracted adipocytes.


2.5 The three steps of the project

The project consisted of the following three steps:

- The translation of the glucose-insulin model to a hierarchical form in *MathModelica*. Modules were created for each organ or part of organ that was included in the model.

- The division of the module describing the glucose utilization by body tissues into two modules; one describing the muscle and one the adipose tissue glucose utilization.

- The creation of a detailed module of the adipose tissue. The parameters of the module were optimized, using cellular experimental data and the input-output profile of the original module. The new module was then inserted in the whole-body model, and effects and new possibilities were evaluated.
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3 Results

The resulting whole-body model with a mechanistic detailed module of the adipose tissue can be used to study type 2 diabetes and related diseases. With this model it is possible to simulate whole-body effects of changes on the cellular level. This is of interest, for example, in the development of drugs. The reason is that drugs act on the cellular level, but that the clinical interest lies on the whole-body level. In this chapter the results from the three main steps of the project, leading to such a hierarchical model, are presented.
3.1 First step: Translation of the whole-body model to a hierarchical format

In the creation of the top level version of the model, the main decisions concerned what should be viewed to the user. Most organs were selected as modules to be shown in the top level, but for some organs several modules were created. For example, the liver both produces glucose and receives insulin from the pancreas to degrade and pass further, and these behaviors were modeled in two different modules.

Figure 3.1.
The top level of the glucose-insulin model. Each small square represent a module with underlying equations.
The top level of the hierarchical model illustrates the model and gives an overview (Figure 3.1). Each square represents a module and the arrows show flows (completed lines) and signals (dotted lines) of glucose and insulin. Background shapes indicate which modules that can be found in the same organ.

The modules were created using the equations from the Dalla Man model. These equations can be found in Appendix 1. The states were modeled as substances. For the reactions, matching reaction equations were created using the BioChem library. An example of a module of the amount of insulin in the liver can be seen in Figure 3.2.

**Figure 3.2.**
A simple module of the amount of insulin in the liver from the Dalla Man model. The right circle represent the amount of insulin in the liver, the left one take care of insulin going out of the system and the above in between represent a calculated signal that affect the degradation. The arrows represent the reactions. The small crosses are the connections to the other modules.
In appendix 3 all the modules, together with their top level symbols, can be found. The modules are:

- Gastrointestinal tract – the glucose digestion through the stomach and intestine
- Plasma glucose – the glucose amount in the plasma, and the glucose utilized by the red blood cells and the brain
- Plasma insulin – the insulin amount, and degradation in the plasma
- Liver insulin – the insulin amount, and degradation in the liver
- Endogenous glucose production (liver) – the glucose production in the liver that depends on insulin levels in the plasma
- Beta cell (pancreas) – the insulin production by beta cells in the pancreas
- Tissue glucose – the glucose that is located out in the tissues
- Tissue utilization (muscle and adipose tissues) – the glucose that is utilized by muscle and adipose tissue that depends on insulin levels in the plasma
- Renal excretion (kidneys) – Glucose excreted to the kidneys that only occur in persons with type 2 diabetes

This first hierarchical model was tested to see that the simulations gave the same results as the Dalla Man model. The simulated graphs from the first hierarchical model are shown in Figure 3.3. The flat model and the article by Dalla Man worked as a reference in the validation of the model. The graphs in Figure 3.3 correspond well with the simulations in the article by Dalla Man et al. [2007]. The authors were also contacted to receive their simulation file of the model. Once obtained, comparisons were also made with equivalent result.
Figure 3.3.
Simulations of the whole-body model; glucose in plasma, insulin in plasma, endogenous glucose production, rate of appearance from intestine to plasma, glucose utilization and insulin secretion.
3.2 Second step: Division of tissues module into muscle and adipose tissue

The tissues module in the Dalla Man model describes the utilization of glucose. The utilization depends on the concentration of insulin and glucose around the cells in the body tissues. The second step in this project was the division of the utilization module into two parts; one describing muscle and one adipose tissue.

Muscle and adipose tissue have the same important glucose transporters; GLUT1 and GLUT4. Other glucose transporters are of lower impact in these tissues [Mueckler 1994]. Different glucose transporters have different $K_m$ values of the Michaelis-Menten reactions. The number of transporters and the number of cells results in a maximal glucose uptake ($V_{\text{max}}$). Literature values of $K_m$ in muscle tissue are 3 mM [Baqué et al. 1998] and 6-9 mM [Laakso et al. 1990] and in adipose tissue 9 mM [Ciaraldi et al. 1979]. Experiments have also shown $K_m$ values of the GLUT4 transporter when inserted in oocytes. Those values are 4.3 mM [Nishimura et al. 1993] and 4.6 mM [Burant and Bell 1992]. The $K_m$ value for muscle and adipose tissue are thus in the same range of 3-9 mM.

The differences in the maximal glucose uptake $V_{\text{max}}$ has also been studied in the literature. In normal state with low insulin levels (before a meal) the glucose uptake by muscle and adipose tissue is 80 % and 20 %, respectively [Gerich 2000]. Measurements in insulin stimulated state inside the human body of the uptake rate have given the results shown in Table 3.1.
### Table 3.1. Glucose uptake by muscle and adipose tissue

<table>
<thead>
<tr>
<th>Adipose tissue (under skin) glucose uptake rate</th>
<th>Muscle tissue glucose uptake rate</th>
<th>Calculated uptake rate by adipose tissue (30 % of body weight)</th>
<th>Calculated uptake rate by muscle tissue (40 % of body weight)</th>
<th>Calculated adipose / muscle tissue as a percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 µmol/kg tissue/min [Virtanen et al. 2002]</td>
<td>56 µmol/kg tissue/min [Virtanen et al. 2002]</td>
<td>5.1 µmol/kg/min</td>
<td>22 µmol/kg/min</td>
<td>19 % / 81 %</td>
</tr>
<tr>
<td>10 µmol/kg tissue/min [Virtanen et al. 2005]</td>
<td>33 µmol/kg tissue/min [Virtanen et al. 2005]</td>
<td>3 µmol/kg/min</td>
<td>13 µmol/kg/min</td>
<td>19 % / 81 %</td>
</tr>
<tr>
<td>19 µmol/kg tissue/min [Viljanen et al. 2009]</td>
<td>48 µmol/kg tissue/min [Viljanen et al. 2009]</td>
<td>5.7 µmol/kg/min</td>
<td>19 µmol/kg/min</td>
<td>23 % / 77 %</td>
</tr>
<tr>
<td>22 µmol/kg tissue/min [Viljanen et al. 2009]</td>
<td>65 µmol/kg tissue/min [Viljanen et al. 2009]</td>
<td>6.6 µmol/kg/min</td>
<td>26 µmol/kg/min</td>
<td>20 % / 80 %</td>
</tr>
</tbody>
</table>

The glucose uptake rate in the human body for the tissues are thus approximately 20 % for adipose tissue and 80 % for muscle tissue both in insulin stimulated state, i.e. after a meal, and when insulin is at basal levels.

The results of the literature studies are:

- The $K_m$ values describing the glucose uptake for muscle and adipose tissue are in the same range (3-9 mM).
- $V_{max}$ for the glucose uptake rate in tissues are divided so that approximately 80 % is used by muscle tissue and 20 % by adipose tissue.

The division of the tissues was module performed in a simple manner. The same equations were kept, with 20 % to adipose tissue and 80 % to muscle tissue. The only validation needed was thus a single simulation of the glucose uptakes from the tissue.
Hierarchical modeling of diabetes

modules created and to control that the rest of the simulated flows and concentrations were not affected.

The expressions for the muscle and adipose tissue utilization after division became:

\[
U_{\text{muscle}}(t) = 0.8 \cdot \frac{V_m(X(t)) \cdot Glu_{\text{tissues}}(t)}{K_m + Glu_{\text{tissues}}(t)} \quad \text{and} \quad U_{\text{fat}}(t) = 0.2 \cdot \frac{V_m(X(t)) \cdot Glu_{\text{tissues}}(t)}{K_m + Glu_{\text{tissues}}(t)}
\]

All parameters were kept the same as in the initial original model.

Graphs showing the simulations of the muscle and adipose tissue utilization can be found in Figure 3.4.

![Figure 3.4](image)

*Figure 3.4.* Utilization from muscle (–) and adipose tissue (--) after division.
3.3 Third step: Creation and optimization of a detailed adipose tissue module

The major challenge in this project was to create a detailed adipose tissue module, optimize the parameters to the module, and insert it into the hierarchical model.

3.3.1 Experimental data

The experimental data used in the optimization were dose response data for IR (n= 5), IRS1 (n= 4) and PKB (n= 5). All the data was taken from Danielsson et al. [2005], where human adipocyte experiments with insulin concentrations of 0-100 nM had been performed.

Glucose uptake rate data were also used in optimization and available from the article Danielsson et al. [2005], but the measurements were performed at lower glucose concentrations than physiological. The existing data were from experiments with a concentration of 0.05 mM around the cells. Physiological concentration of plasma glucose in the human body is approximately 3-8 mM [Wolever et al. 1997] and the concentration near the adipocytes is about the same. The maximal glucose uptakes in the experiments from the article were only 19-214 nmol/min per liter cells (= 0.001-0.01 mg/min per kg of a 78 kg person with 21 L adipose tissue). The division of adipose (20%) and muscle tissue (80%) in the Dalla Man model gave a maximal glucose uptake rate by the adipose tissue of 1 mg/min per kg (Figure 3.5). The maximal uptake rate in the experiments was thus more than 100-fold lower than in the model.

Because of the low glucose concentration and following low uptake rate in previous experiments new experiments were performed. Anita Öst and Cecilia Johansson in the Strålfors group performed measurements with 0.5 mM glucose (10-fold), 2.5 mM (50-fold) and 5 mM (100-fold).
The calculated data from two different experiments on the glucose uptake rate by adipocytes surrounded with 0.05 mM insulin are found in Table 3.2. The measured unit of the radioactive decay from the radio labeled glucose (2-deoxy-D-[H\textsuperscript{3}]-glucose) taken up by the adipocytes was decays/min. The experiments were performed on adipocytes from two different persons. The first experiment show a 2-fold increase in the glucose uptake rate with higher insulin concentration and the second experiment show a more than 3-fold increase. There is a huge variation in insulin stimulated glucose uptake between individuals. In the experiments described in the article Danielsson et al. [2005], the maximal glucose uptake varies 10-fold between individuals.

**Table 3.2: Glucose uptake experiments with 0.05 mM glucose**

<table>
<thead>
<tr>
<th>Insulin, nM</th>
<th>Normal 1, decays/min</th>
<th>Normal 1 glucose uptake, mg/min</th>
<th>Normal 2, decays/min</th>
<th>Normal 2 glucose uptake, mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1234</td>
<td>0.52</td>
<td>1635</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>1154</td>
<td>0.48</td>
<td>3394</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>1716</td>
<td>0.71</td>
<td>4476</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>1988</td>
<td>0.82</td>
<td>5130</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>2201</td>
<td>0.92</td>
<td>4879</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2753</td>
<td>1.15</td>
<td>5656</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>2367</td>
<td>0.99</td>
<td>5289</td>
</tr>
<tr>
<td>No cells</td>
<td>181840</td>
<td></td>
<td></td>
<td>153240</td>
</tr>
</tbody>
</table>
The glucose uptake rates by adipocytes surrounded by 0.5 mM glucose were higher than those with 0.05 mM glucose (Table 3.3). The increase with insulin stimuli was about 3-fold, i.e. a normal response. The higher glucose concentration is closer to physiological and was therefore used in the optimization process.

**Table 3.3: Glucose uptake experiments with 0.5 mM glucose**

<table>
<thead>
<tr>
<th>Insulin, nM</th>
<th>10 times, decays/min</th>
<th>10 times glucose uptake, mg/min</th>
<th>10 times glucose uptake, mg/min per kg (data used in optimization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>439</td>
<td>3.4</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>884</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>940</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>1013</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>1293</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1323</td>
<td>10.6</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1380</td>
<td>10.6</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1324</td>
<td>10.6</td>
</tr>
<tr>
<td>No cells</td>
<td></td>
<td>187490</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4 shows the results from glucose uptake rate measurements by adipocytes from the same person surrounded with 2.5 mM (physiological concentration) and 0.05 mM glucose. The latter worked as a reference. The experiment with 0.05 mM glucose shows an almost 2.5-fold increase in glucose uptake rate, but with higher glucose concentration the increase was only 1.5-fold. The result could thus not be used in the optimization. The reason for the low response could be that the cells became saturated with glucose.
Table 3.4: Glucose uptake experiments with 2.5 mM glucose compared to 0.05 mM

<table>
<thead>
<tr>
<th>Insulin, nM</th>
<th>Normal, Decays/min</th>
<th>Normal glucose uptake, mg/min</th>
<th>50 times glucose, Decays/min</th>
<th>50 times glucose uptake, mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1757</td>
<td>0.54</td>
<td>2995</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>2020</td>
<td>0.66</td>
<td>3331</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>2209</td>
<td>0.72</td>
<td>3279</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>3121</td>
<td>1.02</td>
<td>3507</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>3035</td>
<td>0.99</td>
<td>4137</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3820</td>
<td>1.24</td>
<td>4240</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>3670</td>
<td>1.19</td>
<td>4670</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>3981</td>
<td>1.29</td>
<td>4550</td>
</tr>
<tr>
<td>No cells</td>
<td></td>
<td>180734</td>
<td></td>
<td>864395</td>
</tr>
</tbody>
</table>

3.3.2 Creation of detailed adipose tissue module

We created a rather simple model of the glucose-insulin system in adipocytes. The aim was to create a minimal working model based on knowledge from the group and data from measurements. The existing data was insulin effects on glucose uptake in adipocytes and on the three proteins IR, IRS1 and PKB. The glucose transporters GLUT1 and GLUT4 were also included in the model, but there were no data of them available. First, the model was created in the software MATLAB. MATLAB was chosen since that the optimization was performed there. The model is viewed in Figure 3.6.
Results

The model equations based on Figure 3.6 was the first model approach. The linear equations were as follows:

\[
\begin{align*}
\frac{d}{dt}(IR_{\text{insulin}}) &= v_{f1} - v_{b1} \\
\frac{d}{dt}(IRS1) &= v_{f2} - v_{b2} \\
\frac{d}{dt}(PKB) &= v_{f3} - v_{b3} \\
\frac{d}{dt}(GLUT4_{\text{M}}) &= v_{f4} - v_{b4} \\
\frac{d}{dt}(GLUT1) &= v_{f5} \\
\end{align*}
\]

\[
\begin{align*}
v_{f1} &= k_{f1}(IR_{\text{tot}}-IR_{\text{insulin}})ins \\
v_{f2} &= k_{f2}(IRS1_{\text{tot}}-IRS1)*IR_{\text{insulin}} \\
v_{f3} &= k_{f3}(PKB_{\text{tot}}-PKB)*IRS1_P \\
v_{f4} &= k_{f4}(GLUT4_{\text{tot}}-GLUT4_{\text{M}})*PKB_P \\
v_{f5} &= k_{f5}(GLUCOSE_{\text{tissue}})*GLUT4_{\text{M}}+glut_1*(GLUCOSE_{\text{tissue}}) \\
\end{align*}
\]

Figure 3.6.
The detailed model of adipocytes. Amounts of insulin and glucose near the adipose tissues are the input signals and glucose utilized is the output signal.
Hierarchical modeling of diabetes

\[ \begin{align*}
v_{b1} &= k_{b1} \cdot IR_{\text{insulin}} \\
v_{b2} &= k_{b2} \cdot IRS1_P \\
v_{b3} &= k_{b3} \cdot PKB_P \\
v_{b4} &= k_{b4} \cdot GLUT4_M \\
\end{align*} \]

The linear model was then extended to include saturation with respect to insulin and glucose (on both GLUT1 and GLUT4) concentrations, according to classical Michaelis-Menten statements. The Michaelis-Menten constants were optimized. A whole-body constant \((k_{\text{wholebody}})\) was included to scale up the adipocyte model to the model of the adipose tissue of the whole-body. The final model of the adipose tissue glucose uptake with saturations consisted of the following equations:

\[ \begin{align*}
d/dt(IR_{\text{insulin}}) &= v_{f1} - v_{b1} \\
d/dt(IRS1_P) &= v_{f2} - v_{b2} \\
d/dt(PKB_P) &= v_{f3} - v_{b3} \\
d/dt(GLUT4_M) &= v_{f4} - v_{b4} \\
\end{align*} \]

\[ \begin{align*}
v_{f1\_dynamic} &= k_{f1}\left((IR_{\text{tot}} - IR_{\text{insulin\_dynamic}}) \cdot I \right) / (K_{m1} + I) \\
v_{f2\_dynamic} &= k_{f2} \cdot (IRS1_{\text{tot}} - IRS1_{P\_dynamic}) \cdot IR_{\text{insulin\_dynamic}} \\
v_{f3\_dynamic} &= k_{f3} \cdot (PKB_{\text{tot}} - PKB_{P\_dynamic}) \cdot IRS1_{P\_dynamic} \\
v_{f4\_dynamic} &= k_{f4} \cdot (GLUT4_{\text{tot}} - GLUT4_{M\_dynamic}) \cdot PKB_{P\_dynamic} \\
v_{f5\_dynamic} &= k_{f5} \cdot (G_t) \cdot GLUT4_{M\_dynamic} / (K_{m4} + G_t) + \text{glut}_1 \cdot (G_t) / (K_{m1} + G_t) \\
v_{b1\_dynamic} &= k_{b1} \cdot IR_{\text{insulin\_dynamic}} \\
v_{b2\_dynamic} &= k_{b2} \cdot IRS1_{P\_dynamic} \\
v_{b3\_dynamic} &= k_{b3} \cdot PKB_{P\_dynamic} \\
v_{b4\_dynamic} &= k_{b4} \cdot GLUT4_{M\_dynamic} \\
\text{glucose\_uptake} &= k_{\text{wholebody}} \cdot v_{f5\_dynamic} \\
\end{align*} \]

In the end a total of 19 parameters were optimized. The parameter names and values achieved in the optimization are shown in Table 3.5.
Table 3.5: The parameters in the optimization process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR_tot</td>
<td>23000000000000</td>
<td>amount</td>
<td>total of IR in one cell</td>
</tr>
<tr>
<td>IRS1_tot</td>
<td>15300000000</td>
<td>amount</td>
<td>total of IRS1 in one cell</td>
</tr>
<tr>
<td>PKB_tot</td>
<td>16000000</td>
<td>amount</td>
<td>total of PKB in one cell</td>
</tr>
<tr>
<td>GLUT4_tot</td>
<td>76000000</td>
<td>amount</td>
<td>total of GLUT4 in one cell</td>
</tr>
<tr>
<td>K_f1</td>
<td>2.3</td>
<td>/min</td>
<td>rate constant, IR</td>
</tr>
<tr>
<td>K_f2</td>
<td>0.039</td>
<td>/min</td>
<td>rate constant, IRS1</td>
</tr>
<tr>
<td>K_f3</td>
<td>1.3</td>
<td>/min</td>
<td>rate constant, PKB</td>
</tr>
<tr>
<td>K_f4</td>
<td>0.019</td>
<td>/min</td>
<td>rate constant, GLUT4</td>
</tr>
<tr>
<td>K_f5</td>
<td>13.8</td>
<td>/min</td>
<td>rate constant, glucose uptake</td>
</tr>
<tr>
<td>K_b1</td>
<td>51000000000</td>
<td>/min</td>
<td>rate constant, IR</td>
</tr>
<tr>
<td>K_b2</td>
<td>5600000000</td>
<td>/min</td>
<td>rate constant, IRS1</td>
</tr>
<tr>
<td>K_b3</td>
<td>2280000000</td>
<td>/min</td>
<td>rate constant, PKB</td>
</tr>
<tr>
<td>K_b4</td>
<td>3700000000</td>
<td>/min</td>
<td>rate constant, GLUT4</td>
</tr>
<tr>
<td>glut_1</td>
<td>0.0670</td>
<td>/min</td>
<td>constant for the maximal glucose uptake rate by GLUT1</td>
</tr>
<tr>
<td>K_wholebody</td>
<td>2.4</td>
<td>dimen.</td>
<td>constant for translating cell glucose uptake to whole-body uptake</td>
</tr>
<tr>
<td>Km1</td>
<td>0.009</td>
<td>mg/kg</td>
<td>Michaelis-Menten constant (GLUT1)</td>
</tr>
<tr>
<td>Km4</td>
<td>4000</td>
<td>mg/kg</td>
<td>Michaelis-Menten constant (GLUT4)</td>
</tr>
<tr>
<td>Km</td>
<td>1480</td>
<td>pM</td>
<td>Michaelis-Menten constant (insulin binding to IR)</td>
</tr>
<tr>
<td>K_volume</td>
<td>14</td>
<td>mg/kg per mM</td>
<td>volume available for glucose near the adipocytes</td>
</tr>
</tbody>
</table>

Many different parameter sets gave almost the same simulations, and the parameters were not identifiable. This is the reason for the non-realistic parameter values. However, the point in this project was to include a detailed model of the adipose tissue in the whole-body model, not to find realistic parameter values.

The simulations of the model with the found parameter values, compared to the experimental data, are shown in Figure 3.7. As can be seen in the figure, the simulations correspond well to the experimental data.
Figure 3.7.
From top left the simulated dose response curves for IR, IRS1 and PKB compared to experimental data (*). For GLUT4 there are no data. Bottom left is the glucose uptake rate for different insulin concentrations and bottom right the glucose uptake rate over time after a meal.
3.3.3 Integration of adipose tissue module

The model was also created as a module in MathModelica (Figure 3.8). The acceptable parameter set was included and the module was integrated in the whole-body model.

After insertion of the detailed adipose tissue model in the whole-body model in MathModelica we first wanted to check that simulations of the adipose tissue module gave similar results compared to the MATLAB optimization simulations.

The glucose uptake rate by the newly developed adipose tissue module, compared to 20 % of the existing uptake rate, can be found in Figure 3.9. As can be seen, the new module utilizes glucose in a way that is similar to the old one. The difference is that the new module reaches maximum earlier and has a slightly different shape. At this point we
cannot tell which is most correct, since we have no access to dynamical measurements of glucose uptake rate.

We then wanted to check that the new module had negligible effect on the simulations of glucose and insulin flows and concentrations at the whole-body level. Comparisons were made with the model created in step 2, which has adipose and muscle tissue in different modules. In Figure 3.10 the simulations for the whole-body model with the detailed mechanistic adipocyte module is shown. The simulations are almost identical to the previous simulations with the old adipocyte module. This means that we have successfully developed a zoomable module for the adipose tissue, where zooming does not change the overall dynamics, but only adds/removes details within the adipose module.

Figure 3.9.
The glucose uptake rate by the developed adipose tissues module (--) compared to the glucose uptake rate by the old model of the adipose tissue (–)
Figure 3.10.
The original whole-body model (–) compared to the whole-body model with a mechanistic description of the adipose tissue (---). Top: glucose and insulin in plasma, middle: glucose from intestine and glucose production, down: muscle utilization and secretion of insulin.
3.3.4 Whole-body effects of cellular changes

To further validate the model and to see the potential of a hierarchical whole-body model, the whole-body effect of a change on the cellular level in the adipocyte was examined. The IRS1 protein was made 5 times less sensitive to IR, i.e. to insulin. This lowers the insulin signaling cascade and the insulin dependent glucose uptake. Less sensitive IRS1 protein is one of the mechanisms that make the tissue insulin resistant and it is a common problem in type 2 diabetes [Danielsson 2007]. A simulation of the glucose uptake rate by the adipose tissue after the change can be found in Figure 3.11. The figure shows an approximately 2-fold lower glucose uptake rate, the same size as the original perturbation.

![Figure 3.11.](image)

*Figure 3.11.*
*The effect on the glucose uptake by adipose tissue when IRS1 is 5 times less sensitive to insulin (---). The glucose uptake by adipose tissue from step 2 in the project work as a reference (–).*

Despite this dramatically lower glucose uptake rate on the cellular level, the effect on the amount of glucose in the plasma is almost none, as can be seen in Figure 3.12. Both the glucose production in the liver and the utilization by muscle tissue on the other hand becomes slightly higher. Insulin in plasma and production of insulin lowers when the IRS1 protein becomes less sensitive to insulin. All in all, the effects are rather small, and the reasons and implications of this will be discussed in the next chapter.
Results

Figure 3.12.
From top left there is the effect on glucose in plasma, insulin in plasma, glucose production, insulin production and glucose uptake by muscle when IRS1 is 5 times less sensitive to insulin (---). Whole lines (––) are the references.
4 Discussion

When making a model of a system in the human body it is of interest to make biologists, clinicians and other non-modelers understand it, without insight in the theory behind it. The hierarchical model of the glucose-insulin system in MathModelica is easy to overview thanks to the graphical top layer and underlying modules describing the different organs. More important, perhaps, is that MathModelica is a hierarchical software, which:

a) is designed to facilitate hierarchical modeling

b) make the modeler think in hierarchical terms

An advantage when combining sub-models on different levels into a hierarchical model is that not only interactions on the single level can be studied, but also dynamical interactions between the levels. It should be emphasized that this is not possible by
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simpler approaches, e.g., where the detailed model is simulated first, and the result is included in the top-level simulations, or vice-versa.

In the second step of this project the tissue module was divided in two parts; one describing adipose tissue and one muscle tissue. The division was made in the simplest possible way by setting 20% of the glucose uptake rate to adipose tissue and 80% to muscle tissue. The division was based on literature that showed that both the basal glucose uptake and the insulin stimulated glucose uptake rate were divided like that. No data was found with measurements of the changing glucose uptake rates with time after a meal. Given such information a more sophisticated division could have been made, maybe with completely new expressions. Generally, this division step is important since if the division is not correct, the adipose tissue module created in the next step will be fitted to incorrect whole-body data.

In the model, the glucose uptake by erythrocytes (red blood cells) and the brain are explained with a constant glucose uptake at all times. This is indeed a simplified model of the real situation; all uptake rates vary with glucose concentration. When this glucose uptake is not described correctly, the uptake by muscle and adipose tissue can not be described completely.

Along the same lines, the liver’s glucose uptake is not considered at all in the Dalla Man model. When the uptake from all organs in the body is not included, the existing organs can not be described in a correct way; the rest of the organs must compensate for the non-included liver uptake.

All together this indicates that the utilization of glucose by tissues and other parts in the body can be further developed.

Glucose uptake rate experiments on adipocytes shows a huge individual diversity in the population. Between individuals there can be a 10-fold difference in glucose uptake rate.
This leads to problems. For instance, it is hard to base a model on experiments that varies much, and analyses of the results are difficult to perform. The adipose tissue optimization was based on only one glucose uptake experiment. The reason for this was limited access to data from experiments with a sufficiently high glucose concentration to give a physiologically reliable glucose uptake, which of course is necessary if the sub-models should fit in the whole-body dynamics.

In the earlier experiments they used glucose concentration which did not correspond to physiological concentrations. This was found out during the inclusion of the adipose tissue module in the whole-body model. This exemplifies a benefit obtained from combining the cellular and the whole-body levels, i.e. of using hierarchical modeling; that experimental settings for the subsystems are forced to make sense for the whole-body situation.

When trying to perform those improved glucose uptake experiments at the most physiologically realistic glucose levels, no increase in glucose uptake with increasing insulin concentrations was seen. This was probably due to the fact that the adipocytes became saturated with glucose (to be able to measure all glucose taken up, 2-deoxy-D-glucose, which the cell cannot metabolize, were used). Other possible explanations to the non-responding adipocytes could be that the viscosity of the solution changed when more glucose were added. Physiological glucose concentrations could thus not be used in the optimization.

Many of the parameter values achieved in the optimization, such as values of the rate and total amount, are difficult to validate. For instance, the parameters $K_{m1}$ and $K_{m4}$ have the same unit and $K_{m1}$ should be around 2 times bigger than $K_{m4}$, according to literature [Burant and Bell 1992]. Here $K_{m1}$ instead is 1000000 times smaller than $K_{m4}$ and the value is low related to the glucose concentration. This indicates that GLUT1 in the model does not depend on the glucose concentration but is constant at all times. The value of $K_{m}$ describes the saturation of IR to insulin. The parameter, $k_{\text{wholebody}}$ was thought to
describe the translation from an adipocyte to the whole-body adipose tissue. The value of the parameter was small which indicates that the adipose tissue module describes the glucose uptake rate of the whole adipose tissue, and not a single adipocyte. The last parameter, \( k_{\text{volume}} \) was used to calculate between mM and mg/kg, the unit used for glucose around tissues in the Dalla Man model. The reason for the optimization of this parameter was that it is hard to know the available volume for the glucose molecules.

The simulated dose response curves of IR, IRS1 and PKB all have similar EC\(_{50}\) values. However, the experiments show more sensitiveness to insulin down in the signal cascade. The cause of the simulation results is probably the linear structure of the model. To make a more agreeable model, nonlinearities and feedbacks must be included, so that the signals can be increased downstream in the cascade, leading to a higher separation of the EC\(_{50}\) values.

The last interesting test was the lowering of the IRS1 protein sensitiveness to IR i.e. insulin in the adipose tissue module. The result on cellular level was a dramatic decrease in the glucose uptake rate by the adipose tissue. The reason for this is that insulin controls most of the glucose uptake rate. On a whole-body level increased amounts of glucose in the plasma were expected because the glucose utilized by the adipose tissue decreased. This resulted in almost no change in plasma glucose, and the reason seems to be that the other organs compensates the missing glucose uptake. The muscle tissue utilizes more glucose and the liver produces less glucose. Another effect when lowering the sensitiveness is that the insulin production and the insulin in plasma increase. The body wants to get rid of the higher glucose concentration in the plasma and produces more insulin to do that. This model hence describes a malfunctioning adipose tissue in an otherwise healthy person. A type 2 diabetic person cannot compensate for decreased glucose uptake by the tissues and the glucose concentration in the plasma increases over normal levels.
In respect to this, one last important shortcoming with the whole-body model is that feedback signals from tissues to the rest of the body are missing; the only included metabolites and hormones are glucose and insulin, respectively. In reality both the muscle and adipose tissue sends out signals that the rest of the body respond to. The effect of this shortcoming is that the model underestimates the importance of the adipose tissue, as can be seen in the small whole-body effects in Figures 3.8.

In summary, the whole-body model with an integrated mechanistic module of the adipose tissue can describe experimental measurement performed in vitro on different proteins and glucose uptake rate in adipocytes as well as whole-body calculations of glucose uptake rate after a meal. This is a first model with this ability, starting from a whole-body model, and this pilot-study has thus shown that these kinds of hierarchical extension are feasible.
5 Conclusions

Type 2 diabetes is revolved around the complex glucose-insulin system. The glucose-insulin system involves the cellular level, where cells produce and take up the substances, but also the whole-body level, where the substances circulate between the organs. To combine these levels and ultimately to understand the full complexity of the system, hierarchical modeling is essential. In this project a pilot study of hierarchical modeling of the glucose-insulin system has been performed.

The linking of the whole-body level with underlying tissue and cellular levels has here been performed using the software MathModelica, which support hierarchical modeling. In MathModelica the created modules of the organs can be zoomed in and replaced with more complex ones with cellular details included.
Experimental data and knowledge of adipocytes, as well as data and knowledge of the whole-body level, were crucial to develop the hierarchical model. As a consequence of the hierarchical modeling approach, problems with previous experimental setups for adipocytes were identified and new experiments were performed. This has also led to more long-term changes in our experimental ambitions, which clearly exemplifies the benefit of hierarchical modeling; it forces all contributors to think about how to best align their experimental set-up to fit into the understanding of the whole system.

The parameter optimization was doubly constrained to make the module fit both experimental data and the rest of the whole-body model.

All the three steps in this project: creation of a hierarchical whole-body model in MathModelica, division of the tissues module, and inclusion of a detailed adipose tissue module, have been successfully carried out, and found out to be feasible.

In this project the strength and potential of hierarchical modeling have been demonstrated by the simulation of the whole-body effects of a change on the cellular level. This clearly opens the door towards a new kind of research, yielding a more comprehensive and internally consistent understanding of type 2 diabetes.
6 Recommendations

This thesis is just a proof-of-principle study and it is hence just a first step. Here follows a few suggestions of the next steps one might take in the development of this model. For example, one could replace more modules with simple descriptions with detailed mechanistic models. The adipocyte module could be further developed; one could also include other metabolites and hormones in the model; and one could make more measurements under new conditions at the whole-body level.
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Replacement of more modules with mechanistic ones

The other modules in the whole-body model could be replaced with more detailed mechanistic models of the systems as well. Then, knowledge from other research teams around the world is needed. For example, detailed models of the insulin secretion from beta cells have been performed by numbers of research teams. The models of the other modules could be fitted to and integrated in the whole-body model, in a similar manner as has been done in this project.

Inclusion of liver glucose utilization

The liver utilizes a lot of glucose. This is not included in the model. To describe the utilization by muscle and adipose tissues correct, all of the other utilizations in the body must be included in the model. In the current state of the model, the muscle and adipose tissue compensates for the non-existing liver glucose utilization.

More details in the adipocyte module

The adipocyte module developed in this project is far from a correct description of the interactions taking place in an adipocyte in between the insulin signal and the glucose uptake. More proteins and specific phosphorylations could be included, location needs to be taken into account etc. Apart from this insulin signaling network, more processes taking place in adipocytes are of interest. One important part is that adipocytes maintain control of free fatty acid and triglyceride levels in the plasma. Adipose tissue also act as an important endocrine organ by producing hormones such as leptin, resistin and the cytokine TNFα [Kershaw and Flier 2004].

Inclusion of other metabolites and hormones

Insulin and glucose are not the only hormones and metabolites of interest when studying the glucose-insulin system. Other hormones include for example glucagon, fatty acids and adipokines. Inclusions of more substances in the model are complicated and require
structural changes and new equations. Nevertheless, that is essential if a true picture of the role of various organs should be obtained. For instance, currently, the importance of the adipose tissue is most likely underestimated.

**Measurements under other conditions**

Measurements on glucose and insulin levels, as well as measurements on other hormones and metabolites could be done under other conditions, for example during starvation or during exercise. The more fundamentally different types of measurements the model can explain, the closer the model is to achieving a reliable and accurate description of the important implications in glucose homeostasis and type 2 diabetes.
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References


Dalla Man model equations

The Dalla Man model equations are presented here on the following pages. First the equations describing the concentration of glucose and insulin in the plasma (Glucose kinetics and Insulin kinetics) and then the equations describing flows of glucose and insulin between the involved organs (Glucose Rate of Appearance, Endogenous Glucose Production, Glucose Utilization, Insulin Secretion and Glucose Renal Excretion). All these equations together build up the Dalla Man model. The equations can also be found in Dalla Man et al. [2007] and in Dalla Man et al. [2006].

The parameter values to the left correspond to a healthy person, and the values to the right in italic correspond to a person with type 2 diabetes.
Appendix 1: Dalla Man model equations

**Glucose kinetics**

\[
\begin{align*}
\dot{G}_p(t) &= EGP(t) + Ra(t) - U_{ii}(t) - E(t) - k_1 \cdot G_p(t) + k_2 \cdot G_i(t) \\
\dot{G}_i(t) &= -U_{id}(t) + k_1 G_p(t) - k_2 G_i(t) \\
G(t) &= \frac{G_p}{V_G} \\
G_p(0) &= G_{pb} \\
G_i(0) &= G_{ib} \\
G(0) &= G_b
\end{align*}
\]

<table>
<thead>
<tr>
<th>( V_G )</th>
<th>1.88</th>
<th>1.49</th>
<th>dl/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>0.065</td>
<td>0.042</td>
<td>/min</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>0.079</td>
<td>0.071</td>
<td>/min</td>
</tr>
</tbody>
</table>

- \( G_p \): glucose mass in plasma and rapidly equilibrating tissues
- \( G_i \): glucose mass in slowly equilibrating tissues
- \( G \): plasma glucose concentration
- \( EGP \): endogenous glucose production
- \( Ra \): glucose rate of appearance in plasma
- \( E \): renal excretion
- \( U_{ii} \): insulin independent utilization (erythrocytes and brain)
- \( U_{id} \): insulin dependent utilization (muscle and adipose tissue)
- \( V_G \): distribution volume of glucose
Appendix 1: Dalla Man model equations

**Insulin kinetics**

\[
\begin{align*}
\dot{I}_l(t) &= -m_1 \cdot I_l(t) - m_3(t) \cdot I_l(t) + m_2 \cdot I_p(t) + S(t) \\
\dot{I}_p(t) &= -m_2 \cdot I_p(t) - m_4 \cdot I_p(t) + m_1 \cdot I_l(t) \\
I(t) &= \frac{I_p}{V_I} \\
I_l(0) &= I_{lb} \\
I_p(0) &= I_{pb} \\
I_l(0) &= I_b \\
HE(t) &= -m_5 \cdot S(t) + m_6 \\
HE(0) &= HE_b \\
m_3 &= \frac{HE(t) \cdot m_1}{1 - HE(t)}
\end{align*}
\]

<table>
<thead>
<tr>
<th>(V_I)</th>
<th>0.05</th>
<th>0.04</th>
<th>l/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(m_1)</td>
<td>0.190</td>
<td>0.379</td>
<td>/min</td>
</tr>
<tr>
<td>(m_2)</td>
<td>0.484</td>
<td>0.673</td>
<td>/min</td>
</tr>
<tr>
<td>(m_4)</td>
<td>0.194</td>
<td>0.269</td>
<td>/min</td>
</tr>
<tr>
<td>(m_5)</td>
<td>0.0304</td>
<td>0.0526</td>
<td>min*kg/pmol</td>
</tr>
<tr>
<td>(m_6)</td>
<td>0.6471</td>
<td>0.8118</td>
<td>dimensionless</td>
</tr>
<tr>
<td>(HE_b)</td>
<td>0.6</td>
<td>0.6</td>
<td>dimensionless</td>
</tr>
</tbody>
</table>

- \(I_p\): insulin mass in plasma
- \(I_l\): insulin mass in liver
- \(I\): plasma insulin concentration
- \(V_I\): distribution volume of insulin
- \(HE\): hepatic extraction (insulin flux which leaves the liver irreversibly divided by the total insulin flux)
- \(S\): insulin secretion
Appendix 1: Dalla Man model equations

\textbf{Glucose Rate of Appearance (gastrointestinal tract)}

\[
\begin{align*}
Q_{sto}(t) &= Q_{sto1}(t) + Q_{sto2}(t) \\
\dot{Q}_{sto1}(t) &= -k_{gri} \cdot Q_{sto1}(t) + D \cdot dirac(t) \\
\dot{Q}_{sto2}(t) &= -k_{empt} \cdot Q_{sto1}(t) \cdot Q_{sto2}(t) + k_{gri} \cdot Q_{sto1}(t) \\
\dot{Q}_{gut} &= -k_{abs} \cdot Q_{gut}(t) + k_{empt} \cdot Q_{sto2}(t) \\
Ra(t) &= \frac{f \cdot k_{abs} \cdot Q_{gut}(t)}{BW} \\
\end{align*}
\]

\[
Q_{sto}(0) = Q_{sto1}(0) = Q_{sto2}(0) = Q_{gut}(0) = Ra(0) = 0
\]

\[
k_{empt}(Q_{sto}) = k_{\text{min}} + \frac{k_{\text{max}} - k_{\text{min}}}{2} (\tanh(a \cdot (Q_{sto} - b \cdot D)) - \tanh(c \cdot (Q_{sto} - d \cdot D)) + 2)
\]

| \(k_{\text{max}}\) | \(0.0558\) | \(0.0465\) | /min |
| \(k_{\text{min}}\) | \(0.0080\) | \(0.0076\) | /min |
| \(k_{\text{abs}}\) | \(0.057\) | \(0.023\) | /min |
| \(k_{gri}\) | \(0.0558\) | \(0.0465\) | /min |
| \(f\) | \(0.90\) | \(0.90\) | dimensionless |
| \(a\) | \(0.00013\) | \(0.00006\) | /mg |
| \(b\) | \(0.82\) | \(0.68\) | dimensionless |
| \(c\) | \(0.00236\) | \(0.00023\) | /mg |
| \(d\) | \(0.010\) | \(0.09\) | dimensionless |

\(Q_{sto}\) amount of glucose in the stomach  
\(Q_{sto1}\) solid phase  
\(Q_{sto2}\) liquid phase  
\(Q_{gut}\) mass of glucose in the intestine  
\(BW\) body weight  
\(D\) amount of ingested glucose
Appendix 1: Dalla Man model equations

Endogenous Glucose Production (liver)

\[ EGP(t) = k_{p1} - k_{p2} \cdot G_p(t) - k_{p3} \cdot I_d(t) - k_{p4} \cdot I_{po}(t) \]
\[ EGP(0) = EGP_b \]
\[
\begin{align*}
\dot{I}_1(t) &= -k_i \cdot (I_1(t) - I(t)) \\
\dot{I}_d(t) &= -k_i \cdot (I_d(t) - I_1(t))
\end{align*}
\]

\[ I_1(0) = I_d(t) = I_b \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{p1} )</td>
<td>2.70</td>
<td>3.09</td>
<td>mg/kg/min</td>
</tr>
<tr>
<td>( k_{p2} )</td>
<td>0.0021</td>
<td>0.0007</td>
<td>/min</td>
</tr>
<tr>
<td>( k_{p3} )</td>
<td>0.009</td>
<td>0.005</td>
<td>mg/kg/min per pmol/l</td>
</tr>
<tr>
<td>( k_{p4} )</td>
<td>0.0618</td>
<td>0.0786</td>
<td>mg/kg/min per pmol/kg</td>
</tr>
<tr>
<td>( k_i )</td>
<td>0.0079</td>
<td>0.0066</td>
<td>/min</td>
</tr>
</tbody>
</table>

EGP  endogenous glucose production  
\( I_{po} \) amount of insulin in the portal vein  
\( I_d \) a delayed insulin signal in two compartments  
I  plasma insulin concentration  
\( G_p \) glucose mass in plasma and rapidly equilibrating tissues
Appendix 1: Dalla Man model equations

**Glucose Utilization (muscle and adipose tissue)**

\[ U_{ii}(t) = F_{cns} \]

\[ V_m(X(t)) = V_{m0} + V_{mX} \cdot X(t) \]

\[ \therefore U_{id}(t) = \frac{V_m(X(t)) \cdot G_t(t)}{K_m + G_t(t)} \]

\[ \dot{X}(t) = -p_{2U} \cdot X(t) + p_{2U} \cdot (I(t) - I_b) \]

\[ X(0) = 0 \]

\[ U(t) = U_{ii}(t) + U_{id}(t) \]

<table>
<thead>
<tr>
<th></th>
<th>( F_{cns} )</th>
<th>( I )</th>
<th>mg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{m0} )</td>
<td>2.50</td>
<td>4.65</td>
<td>mg/kg/min</td>
</tr>
<tr>
<td>( V_{mX} )</td>
<td>0.047</td>
<td>0.034</td>
<td>mg/kg/min per pmol/l</td>
</tr>
<tr>
<td>( K_{m0} )</td>
<td>225.59</td>
<td>466.21</td>
<td>mg/kg</td>
</tr>
<tr>
<td>( p_{2U} )</td>
<td>0.0331</td>
<td>0.0840</td>
<td>/min</td>
</tr>
</tbody>
</table>

U \quad total glucose utilization

\( U_{ii} \) \quad insulin independent utilization

\( U_{id} \) \quad insulin dependent utilization

\( G_t \) \quad glucose mass in slowly equilibrating tissues

\( X \) \quad insulin in the interstitial fluid

\( I \) \quad plasma insulin concentration
Insulin Secretion (Beta cells)

\[ S(t) = \gamma \cdot I_{po}(t) \]
\[ \dot{I}_{po}(t) = -\gamma \cdot I_{po}(t) + S_{po}(t) \]
\[ I_{po}(0) = I_{pob} \]

\[ S_{po}(t) = \begin{cases} 
Y(t) + K \cdot \dot{G}(t) + S_b & \text{if } \dot{G} > 0 \\
Y(t) + S_b & \text{if } \dot{G} \leq 0 
\end{cases} \]

\[ \dot{Y}(t) = \begin{cases} 
-\alpha \cdot (Y(t) - \beta(G(t) - h)) & \text{if } \beta \cdot (G(t) - h) \geq -S_b \\
-\alpha \cdot Y(t) - \alpha \cdot S_b & \text{if } \beta \cdot (G(t) - h) < -S_b 
\end{cases} \]

\[ Y(0) = 0 \]

<table>
<thead>
<tr>
<th>(K)</th>
<th>2.30</th>
<th>0.99</th>
<th>pmol/kg per mg/dl</th>
</tr>
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<tbody>
<tr>
<td>(\alpha)</td>
<td>0.050</td>
<td>0.013</td>
<td>/min</td>
</tr>
<tr>
<td>(\beta)</td>
<td>0.11</td>
<td>0.05</td>
<td>pmol/kg/min per mg/dl</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>0.5</td>
<td>0.5</td>
<td>/min</td>
</tr>
</tbody>
</table>

\(h\) the threshold level of glucose above which beta cells initiate to produce new insulin. In the model \(h\) has been set to \(G_b\), the basal glucose concentration.

\(S\) insulin secretion to beta cells

\(I_{po}\) amount of insulin in the portal vein

\(S_{po}\) insulin secretion to the portal vein

\(G_t\) glucose mass in slowly equilibrating tissues

Glucose Renal Excretion

\[ E(t) = \begin{cases} 
{k_{c1}} \cdot (G_p(t) - k_{c2}) & \text{if } G_p(t) > k_{c2} \\
0 & \text{if } G_p(t) \leq k_{c2} 
\end{cases} \]

<table>
<thead>
<tr>
<th>(k_{c1})</th>
<th>0.0005</th>
<th>0.0007</th>
<th>/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{c2})</td>
<td>339</td>
<td>269</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>

\(E\) renal excretion

\(G_p\) glucose mass in plasma and rapidly equilibrating tissues
Appendix 1: Dalla Man model equations
model Dalla_Man_Model

Real G_p(start=178);
Real G_t(start=178);
Real G(start=G_b);
Real EGP;
Real Ra(start=0);
Real U_id;
Real U;
Real E;
Real I_l(start=1.25);
Real I_p(start=1.25);
Real I(start=I_b);
Real S(start=S_b);
Real HE;
Real m_3;
Real Q_sto(start=0);
Real Q_sto1(start=D);
Real Q_sto2(start=0);
Real Q_gut(start=0);
Real k_empt;
Real I_d(start=I_b);
Real I_l(start=I_b);
Real U_idm;
Real U_idf;
Real V_mmax;
Real V_fmax;
Real X(start=0);
Real S_po;
Real I_po(start=3.6);
Real Y(start=0);
Real aa;
Real cc;

parameter Real V_G=1.88,k_1=0.065,k_2=0.079,G_b=95;
parameter Real V_I=0.05,m_1=0.19,m_2=0.484,m_4=0.194,
m_5=0.0304,m_6=0.6471,HE_b=0.6,I_b=25,S_b=1.8;
parameter Real k_max=0.0558,k_min=0.008,k_abs=0.057,
k_gri=0.0558,f=0.9,b=0.82,d=0.01,D=78000,BW=78;
parameter Real k_p1=2.7,k_p2=0.0021,k_p3=0.009,k_p4=0.0618,k_i=0.0079;
parameter Real U_ii=1,V_m0=2.5,V_mX=0.047,K_m0=225.59,
V_f0=2.5,V_fX=0.047,K_f0=225.59,p_2U=0.0331,part=0.2;
pärameter Real K=2.3,alpaha=0.05,bet=0.11,gamma=0.5;
päarameter Real k_e1=0.0005,k_e2=339;
Appendix 2: Dalla Man model code

equation
der(G_p)=max(EGP, 0) + max(Ra, 0) - E - U_ii - k_1*G_p + k_2*G_t;
der(G_t)=(-U_id) + k_1*G_p - k_2*G_t;
G=G_p/V_G;
der(I_1)=(-m_1*I_1) - m_3*I_1 + m_2*I_p + S;
der(I_p)=(-m_2*I_p) - m_4*I_p + m_1*I_1;
I=I_p/V_I;
HE=-(m_5*S) + m_6;
m_3=HE*m_1/(1 - HE);
Q_sto=Q_stol + Q_sto2;
der(Q_stol)=-k_gri*Q_stol;
der(Q_sto2)=(-k emptied*Q_sto2) + k_gri*Q_stol;
der(Q_gut)=(-k_abs*Q_gut) + k emptied*Q_sto2;
Ra=f*k_abs*Q_gut/BW;
k emptied=k_min + (k_max - k_min)/2*(tanh(aa*(Q_sto - b*D)) -
tanh(cc*(Q_sto - d*D)) + 2);
aa=5/2/(1 - b)/D;
cc=5/2/d/D;
EGP=if EGP < 0 then 0 else k_p1 - k_p2*G_p - k_p3*I_d - k_p4*I_po;
der(I_1)=k_1*(I_1 - I);
der(I_d)=k_1*(I_d - I_1);
U idm=V_mmax*G_t/(K_m0 + G_t);
V_mmax=(1 - part)*((V_m0 + V_mX*X));
U idf=V_fmax*G_t/(K_f0 + G_t);
V_fmax=part*(V_f0 + V_fX*X);
U id=U idm + U idf;
der(X)=(-p_2*U*X) + p_2*U*(I - I_b);
U=U_ii + U id;
S=gamma*I_po;
der(I_po)=(-gamma*I_po) + S po;
S po=if der(G_p) > 0 and G > G_b then Y + K*der(G) + S b else Y + S b;
der(Y)=if beta*(G - G_b) < -S b then (-alpha*Y) - alpha*S b else -
alpha*(Y - beta*(G - G_b));
E=if G_p < k e2 then k e1*(G_p - k e2) else 0;
end Dalla_Man_Model;
Appendix 3: Modules of the hierarchical model

Gastrointestinal tract

Plasma glucose
Appendix 3: Modules of the hierarchical model

Plasma insulin

Liver insulin
Appendix 3: Modules of the hierarchical model

Endogenous glucose production (liver)

Beta cell (pancreas)
Appendix 3: Modules of the hierarchical model

Tissue glucose

Tissues utilization (muscle and fat tissue)
Appendix 3: Modules of the hierarchical model

Renal excretion (kidneys)
Appendix 3: Modules of the hierarchical model