Multi-level multi-scale metabolites simulation

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Abstract

Diabetes is a world-wide health problem with 415 millions of people suffering from the disease. Most diabetics are suffering from Type 2 Diabetes, which is preceded by insulin resistance in glucose utilizing tissues, such as adipose, liver, and muscle tissues. Diabetes is diagnosed when the insulin control of the glucose levels fails, which leads to high glucose levels in the blood. To better understand the insulin control of blood glucose, mathematical modeling has been used for many years to simulate the dynamics of glucose and insulin levels in the blood. Models have also been used to understand the intracellular insulin-signaling network in the insulin responding tissues. There have also been attempts to connect models from these different layers of control into a multi-level and multi-scale simulation model. However, to do such connections, several assumptions must be made about the comparability of the data from the different levels. Here, I aim for a deeper understanding of these assumptions and to use more advanced data for glucose uptake dynamics than in earlier work. I used data from the literature for the dynamics of glucose uptake in adipose and muscle tissues and improve the model in several steps to have a better agreement with these data. In particular, I refined the sub-division of the glucose uptake between the organs, to also account for liver uptake, a correction that implied a reduction by 50% for the muscle and adipose tissue glucose uptake. Unlike previous models, the updated model also describes blood flow. Finally, because of the connection to the intracellular level, the model can be used to simulate the response to anti-diabetic drugs.
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1. Introduction

1.1 Glucose and diabetes

A lack of regular exercise or a long-term unhealthy diet can lead to many health problems, for example, high blood pressure, hyperlipidemia, and diabetes. Diabetes is characterized as an abnormal high glucose level in blood. As a world-wide health problem, around 415 millions of people are suffering from diabetes which has been studied for several years to find the cause and effective treatment methods [1].

The glucose level in the blood is normally controlled by hormones secreted by the pancreas (Figure 1.1). For instance, when the glucose level in the blood is abnormally high, the beta cells in the pancreas will secrete the hormone insulin, which promotes the process of glucose consumption in blood so that the glucose level will go down (with ‘-’ symbol in Figure 1.1). Similarly, when the glucose level is too low, the alfa cells in pancreas will release the hormone glucagon, which can stimulate liver to generate more glucose (with ‘+’ symbol in Figure 1.1). Thanks to this control system, the level of glucose in the blood is kept within a narrow range.

However, when this control system fails to control the level of glucose, diabetes can be diagnosed. The reasons for failure could either be in the production of insulin, which happens in Type-1-Diabetes (T1D), or in response to insulin, which happens in Type-2-Diabetes (T2D). T1D is caused by an autoimmune disorder, which means that immune system attacks the cells that make insulin, so that insulin cannot be secreted sufficiently or even cannot be secreted [2]. On the other hand, T2D usually begins with insulin resistance [2]. Insulin resistance is considered as a pathological condition where the cell resists the effects from insulin. In other words, insulin restricted cells cannot respond normally to insulin [2]. The insulin resistance occurs in the intracellular insulin signaling pathway, which is a large network of interacting proteins that regulate cell functions and glucose uptake. Considering that 415 millions of people are suffering from T2D, I implement relative researches about T2D in this thesis.

It is clear from the description above that T2D is high complex. This complexity warrants new tools for analysis that are better suited for dealing with complexity. One such tool is systems biology which is introduced in the next section.
Figure 1.1. Internal auto-control system for blood glucose levels. Alfa-cells secrete glucagon to compensate for the lack of glucose when the glucose level in blood is low; Beta-cells produce insulin to stimulate the consumption of glucose when the glucose level in blood is high. Both mechanisms help maintain the glucose homeostasis.

1.2 Systems biology

In order to simulate the dynamics of biological activities using mathematics, the field of system biology has evolved. Systems biology uses mathematical modeling methods developed in other fields, such as engineering and control theory, to analyze biological data. Systems biology can be seen as the merging of three different fields: biology, mathematics and computer science. With the knowledge from biology, it is possible to have an overview of the biological structure of the target, meanwhile, this assistance from computer power also drastically improves the speed, accuracy, and stability of the simulation of developed models. In short, systems biology is an effective method to study the biological structure with data both theoretically and quantitatively. In practice, the modelling cycle is given in Figure 1.2. At the first step, the experimental data is used as the input to the model to evaluate the hypothesis, if the model cannot explain the data which means the hypothesis is rejected. Then more insights are put into the revision of hypothesis; if the model explains the data, then new predictions are made for more datasets, the iteration lasts until the model is considered valid. [16]
Figure 1.2. Model-based data analysis. First, the experimental data will be used to evaluate the hypothesis through models, if the model cannot explain the data, then the hypothesis is rejected and more insights should be put in; if the model can explain the data, then predictions are made to compare with new data, this iteration lasts until the model is considered valid.

1.3 Models for diabetes

Models of use in this projects have already been developed, such as models for glucose-insulin and insulin signaling pathways. The glucose-insulin model simulates the glucose dynamics under the control of insulin while the insulin signaling pathway is looking into an intracellular level. Two models were chosen to be used in this study and they will be introduced in the following sections.

1.3.1 Glucose-insulin model

A flow chart of the glucose-insulin model, developed in [3], can be seen from Figure 1.3. The input to the glucose-insulin model is a meal which components can be controlled. After the meal is digested, the glucose in the meal will go to the first subsystem which is the glucose system. Glucose system is the place to provide glucose to the whole human body. Generally, there are two resources of glucose to that system, one is the intake food while another is the glucose generated by liver. In this model, glucose is assumed to be utilized by muscle and adipose tissue, and also by the central nervous system. The difference between those utilization terms is that adipose and muscle tissue are insulin-dependent, while central nervous system is insulin-independent.

Other components in this glucose-insulin model are Beta-cell and Insulin system. Beta-cell is the cell existing in pancreas and with the assistance of that cell, insulin can be secreted. The insulin system controls the glucose uptake and glucose production to maintain glucose levels within the normal range. With the corporation of all components in the model, the dynamic glucose change after a meal can be simulated.
1.3.2 Insulin signaling model

The model for intracellular insulin signaling and insulin resistance have been developed in several steps previously [4], [5]. An important mathematical model for insulin signaling pathway, from insulin receptor (IR) to GLUT4 translocation, was published in 2002 [4]. In that model, the author combined the previous insulin-IR and IR recycling models with GLUT4 regulation models. That model also contained the knowledge about mechanisms in the downstream signaling to the glucose uptake. With further developments, one other model which had more detailed theories at constructing insulin signaling was published [5]. The author formulated simple equations for a sigmoidal activation to simulate dose-response behaviors and compared with data from various sources. However, the shortcoming for both [4] and [5] is the lack of dynamic data. Based on other publications of insulin signaling [6] - [14], a mini model of insulin signaling [15], [17] is designed. It is a comprehensive mathematical model that is based on data from human adipocytes gathered from labs for many years. The data includes many important signaling intermediaries such as IR and glucose uptake. With this model, I have taken the knowledge of dynamic intracellular insulin signaling to a new level. An overview of the insulin signaling model from [15], [17] can be found in Figure 1.4.
1.3.3 Combination of glucose insulin model and insulin signaling model

The previous studies on insulin signaling pathway models [15], [17] are mainly based on the in vitro experimental examinations of cells, however, in vivo the studies for humans have not been examined. In order to overcome the lack of studies, a new model from [15] was developed as a hierarchical model of the adipose tissue, which combines the intracellular insulin control of glucose transport in adipocytes with the whole-body glucose homeostasis. In previous studies [15], authors concluded that it was not possible to scale up the experimentally determined glucose uptake by the isolated adipocytes to match the data from in vivo measurements. However, by adding insulin effects on blood flow in adipose tissue and GLUT4 translocation due to cell handling, it was possible to explain the data [15]. The authors used two phases in the combination of models. In the first phase, a minimal model was made to link insulin signaling in the adipocytes with the adipose tissue level. In the second phase, the minimal model was inserted into the glucose-insulin model mentioned in section 1.3.1. The merging divides the utilization of glucose into two parts, muscle and adipose tissue. The values for muscle and adipose tissue are normalized based on the mass distributions in human. The equation of values for both muscle and adipose tissue is given in Equation (1.1) and (1.2). The normalized value for muscle tissue and adipose tissue are 0.2 and 0.8 respectively, which means 20% of the glucose utilization goes to muscle while 80% of glucose utilization goes to adipose tissue. The flow chart for the final combination model is shown in Figure 1.5.

\[
\begin{align*}
\text{Adipose}_{\text{normalization}} &= \frac{\text{Adipose}}{\text{Adipose} + \text{Muscle} + \text{Constant}} \quad (1.1) \\
\text{Muscle}_{\text{normalization}} &= \frac{\text{Muscle}}{\text{Adipose} + \text{Muscle} + \text{Constant}} \quad (1.2)
\end{align*}
\]

where, adipose and muscle indicate the mass of corresponding tissues and constant means the mass of other tissues in the body

![Insulin Signaling Pathways](image)

**Figure 1.4** Scheme of insulin signaling pathways. IRS1, mTORC2, PKB etc., are proteins and intermediates which are not the focus in this thesis. Blue arrows are signaling pathways; green arrow indicates positive feedback signal; gray arrow indicates GLUT4 translocation to the plasma membrane in response to insulin signaling.
Figure 1.5. Hierarchical model for both insulin signaling and whole-body glucose homeostasis. The left side is kept as the whole-body level model; right side is replaced with new developed minimal model in adipose tissue while the muscle tissue part is kept as the whole-body level model.

1.4 Shortcomings with the combination model

Though the combination model provides us with an analysis in both insulin signaling and whole-body glucose homeostasis, the model still has some shortcomings in both the intracellular level and the whole-body level. The minimal intracellular model was developed based on intracellular data from adipocytes, but the combined model has so far not been tested with data from the adipose tissue level. Another issue with the combined model is that the muscle and adipose tissue are assumed utilizing the majority of glucose, for example excluding the liver glucose utilization, and that assumption have not been examined using data for glucose utilization in these tissues. Both of these shortcomings will be addressed in this thesis, using new data and corresponding model development.

1.5 Purpose of the thesis

The purpose of this thesis is to have a deeper understanding on the current combination model and solve the shortcomings mentioned in the last section. First, the current combination model is tested with the assumptions made in 1.3.3 [15]. Second, more data from adipose tissue and muscle tissue level are used to test the model. Detailed research into glucose uptake distribution and cellular glucose uptake behavior will be implemented until the modified model agree with the data good enough. The workflow of this thesis is given in Figure 1.6.

The main result of this thesis is an improved model that is able to fit with new more accurate theories on glucose distribution among all organs, describe blood flow regulation, and a generally improved description of the glucose uptake in adipose tissue. This model implies a deeper systems-level understanding of glucose regulation in humans, both on the whole-body level and on the tissue and intracellular levels.
Figure 1.6 Workflow of the thesis. Firstly, test the combination model, if the simulation agrees with previous assumptions, comparing the result with new datasets. Otherwise modifications are made. Secondly, compare the simulation results with new datasets from recent research, if the simulation is rejected, then modify the model with new theories until the model can explain the datasets.
2. Method

In order to improve the combination model, I have done mathematical model design, parameter optimization and experimental data acquisition. In this section, I start from the basic element in mathematical model—ordinary differential equations to the parameter optimization. Then I go to the new datasets used in this project regarding the measurement methods and scaling process. Finally, the software that is used for simulation is also introduced.

2.1 Ordinary differential equations (ODEs)

The models used in systems biology are mathematical models which contain states, parameters, variables and reactions to mimic things of interest in corresponding biological area. Terms in the mathematical model are denoted in ordinary differential equations (ODEs) which are changing over time. ODE enables us to have a time-varying analysis.

The state in ODE is used to describe the system in a specific time point; reactions are usually shown in the form of product to replace the chemical reactions; parameters and initial values are set to control the scaling and set initial conditions. More examples will be given in the following paragraph to give the reader a better understanding into the concepts.

An example of ODE is given in Figure 2.1, where M and N are states that are changing over time. u, v and w are reactions that connect states. Mathematically, the ODEs for M and N can be denoted as Equation (2.1) and Equation (2.2).

\[
\frac{dM}{dt} = u - v \quad (2.1) \\
\frac{dN}{dt} = v - w \quad (2.2)
\]

where ‘v’ and ‘w’ are reaction rates and they can be written as Equation (2.3) and Equation (2.4):

\[
v = M \cdot k_1 \quad (2.3) \\
w = N \cdot k_2 \quad (2.4)
\]

where \(k_1\) and \(k_2\) are rate parameters.

The values of the parameters are optimized for best agreement between model simulation and data using a cost function and an optimizing algorithm, which will be introduced in the next section.
2.2 Parameter optimization

In order to find the best fitting parameters for the model, a comparison between simulation results and the data is needed. This comparison is commonly referred to as the cost, and calculated as equation (2.5):

\[
\text{cost} = \sqrt{\frac{\sum (y(t) - \hat{y}(t, p))^2}{\sigma^2}} \quad (2.5)
\]

where \(y(t)\) is the measurement and \(\hat{y}(t, p)\) is the simulation with parameter \(p\), \(\sigma\) is the standard deviation of the measurement noise. In the numerator, a square is used to treat positive and negative differences equally. In the denominator, a standard deviation is used to act as a weight factor. A higher standard deviation means a less certain data point, which should have a less contribution to the cost, while a lower standard deviation means a more certain data point.

In order to find the parameter that makes the best fit between measurement and simulation. Simulated annealing is used as the optimization method. An initial parameter value is given and the simulation is compared with measurement. After that, the initial parameter is changed and the simulation is compared again with measurement. The comparison process can be repeated for many times until the smallest cost is found. The parameter corresponds to the smallest cost is considered as the best fit.

2.3 Software support

In this thesis, Matlab_R2015b is used to implement the simulation. Matlab is a multi-paradigm numerical computing environment allowing matrix manipulation, plotting results and algorithm implementation. There are many toolboxes in Matlab for different usages and a toolbox developed for systems biology (‘SBtoolbox’) [31], [32] is used in this thesis.

2.4 Subjects, data measurements and scaling

In order to improve the model and study the glucose-insulin regulation better, more data is needed. In a literature search, tree interesting studies was found for glucose dynamics after a meal [18]-[20]. These studies include tissue specific measurements on both blood flow and tissue glucose uptake. These studies also have similar experimental design (e.g. subjects, meals, measurements method) which provides a comparable result. The data from those papers will be named Data1, Data2 and Data3 in this thesis.

2.4.1 Subjects

The subjects referred in this project are healthy people (i.e. non-diabetic, non-obese). Information for subjects can be seen from Table 1. All the data is extracted as average value to ease the comparison. All the subjects are asked to avoid severe exercise, alcohol and caffeine for 48h before the experiment.
Table 1. Summary of subjects’ information

<table>
<thead>
<tr>
<th>Data</th>
<th>Age (yr)</th>
<th>Body Mass (kg)</th>
<th>Height (m)</th>
<th>Fat Mass (kg)</th>
<th>Muscle Mass (kg)</th>
<th>Body Mass Index (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data1</td>
<td>37 (24~64)</td>
<td>68 (49~91)</td>
<td>1.70 (1.47~1.87)</td>
<td>17(8.2~27)</td>
<td>26(17~31)</td>
<td></td>
</tr>
<tr>
<td>Data2</td>
<td>39 (29~64)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.7</td>
</tr>
<tr>
<td>Data3</td>
<td>39 (35~43)</td>
<td></td>
<td>14.5 (12.5~16.5)</td>
<td>23.5 (21.8~25.2)</td>
<td>23 (22~24)</td>
<td></td>
</tr>
</tbody>
</table>

Body Mass Index = Body weight / Body height²

The empty cells are without available data

2.4.2 Metabolites measurement methods

Blood flow is the first thing that has to be measured since it has to be used to estimate and calculate other metabolites. Catheters are inserted in different parts of the body (e.g. forearm and abdomen) to obtain corresponding blood flow. Due to the different properties of adipose tissue and muscle tissue, two methods are used to measure the corresponding blood flow. Xenon washout method [21] - [24] is used to measure adipose tissue blood flow and mercury strain-gauge plethysmography [25] is used for muscle tissue blood flow measurement. Those methods will be introduced separately.

One thing to mention is, in [18], glucose flux is extracted and used as glucose uptake, since the authors did not directly provide the glucose uptake data. The flux is calculated as the product of its arteriovenous difference and the mean of the blood flow which is measured immediately before and after the sample. In [19], [20], the glucose uptake is calculated in the same way.

- Xenon washout method [24]

Adipose tissue is a very active tissue, not only at metabolic level but also at hormonal level. The normal work of adipose tissue quite relies on the blood flow, since the change of blood flow can alter the metabolic activities. According to the Fick’s equation, the metabolic flux can be calculated as equation (2.6):

\[ EX = BF \times (A - V) \]  

Where EX means exchanged metabolites and hormones, and BF means blood flow. A-V is the arteriovenous difference.

Generally, the blood flow is measured by inserting a radioisotope into the body and observing the decaying or disappearance of it in quantity. The assumption behind that is the radioisotope will decay faster if there is a higher blood flow. Then the blood flow is calculated as the product of several components: k, λ, where k is the exponential rate constant which is a property constant for a specific radioisotope, and it is calculated from the semi-log plot of disappearance; λ is the tissue/blood coefficient of partition of the isotope. So, the blood flow can be denoted as equation (2.7):

\[ BF \left( \frac{100 \text{g of tissue}}{\text{min}} \right) = k \cdot \lambda \cdot 100(g) \cdot 60(s) \]
This theory is only correct when a diffusion coefficient equilibrium is maintained.

In Xenon washout method, Xenon is used as the radioisotope. The reason for choosing Xenon is because of its chemical property. Xenon is an inert chemical which means it is very unlikely to have reactions with surrounding molecules, what is more, Xenon is also lipophilic, and that property makes it ideal for the adipose tissue. Besides, the tissue/blood coefficient of partition of Xenon is quite high, that allows it having a good solution in adipose tissue. What is more, Xenon can be exhaled easily when it passes through lungs so the recirculation is also ensured.

- Strain-gauge plethysmography

Originated from Whitney [25], the plethysmography has become a standard method to measure volume changes in human body for a long time. After being developed and improved, single strain gauge plethysmography becomes to the simplified version of the original method and has been widely used.

The principle behind this technique is not complicated. The relative volume change of a body part is equal to the relative resistance change of the strain gauge which is wrapped around the corresponding area. The exact change in the strain gauge is its circumference, and the change in the length will alter the electrical resistance because the strain gauge is longer and thinner. For different materials, the relationship between changes in length and changes in electrical resistance are different, but by using that specific relationship from each strain gauge, the volume alternation can be calculated. The reason why Xenon washout method is not used for muscle tissue is because the decay curve of Xenon will become multi-exponential gradually, so it is not possible to use Xenon method to measure the extended periods in muscle tissue.

Except for Xenon washout method and strain gauge plethysmography, there are some other methods which can measure the blood flow changes, for example, laser Doppler flowmeter and positron emission tomography. Those methods have their advantages, such us non-invasive, and a better ability to differentiate the individual contribution of different tissue and organs which are between the arterial and venous sampling sites. Positron emission tomography (PET) is used in an extension study in this thesis.

- Positron emission tomography (PET)

As it can be seen from the name, PET has something to do with the positron emission. In fact, this technology is used to measure the decaying signal of radionuclides. Some labelled compound will be made before measurement and specifically for the target cell or molecule, then the labelled compound will be inserted into the body which will attach to the target cell. After a while, the radioactive molecule will start to decay which releases positrons, and that can cause the emission of high-energy photons. Those photons are easy to escape from the body which ensures the safety of this measurement. The detectors will be positioned surrounding the patient to receive those high-energy photons and transfer it into electrical signals.

The reconstruction process of image by using PET is assisted by 3D-image volume, where the intensity of the signal in a single voxel is proportional to the amount of labelled compound, in another word, the amount of target molecules. Relying on that property, PET can be used to measure the concentration of molecules in the human body, and if a sequence of images is taken within a time period, a concentration-time function can be established, with the further assistance of some models, even the rate of some physiological terms can be determined.
2.4.3 Meals

The aim of this thesis is to study the glucose regulation after taking a meal, so to know the actual meal is an important component in the data collection process. Meals are given sometime after the insertion of catheter, with components of protein, carbohydrate, fatty acid and fiber. Measurements are done in dynamic time interval after the intake of meal. Specific introduction of meals in [18], [19], [20] are listed as follows:

- Meals in [18]: A mixed meal [21] estimated from the manufacturer's data and food tables [26] to contain 21.9g of protein, 93g of carbohydrate (of which 45% was simple sugars), 33.5 g of fatty acids and 12g of fiber, giving 3.1MJ (740kcal) of energy, of which 47% came from carbohydrate and 41% from fat. The meal is finished in 20min.
- Meals in [19]: The meal contains 3.1MJ, with 41% of calories from fat and 47% from carbohydrate. [26] The meal is finished in 20min.
- Meals in [20]: A meal is given (730 kcal, 50% carbohydrate, 40% fat, 10% protein) 1 h after catheter insertion and consumed within 20 min [27]. The meal contains 103 g of carbohydrate (of which 38% were simple sugars), 35 g of fat, and 25 g of protein.

2.4.4 Data scaling

The unit of glucose uptake is not unified in different researches. In this project, the unit of glucose uptake is mg/kg/min, which stands for the amount of glucose uptake/body weight/minute. In order to compare the data extracted from other papers with our simulation results, those data needs to be scaled at first. The unit from other reference papers is $\mu$mol/min/100ml. Comparing those units, the only same term is the time, so both glucose amount and tissue volume should be scaled to new units.

Starting with the glucose amount, due to the chemical components, glucose can be denoted as $C_6H_{12}O_6$, meaning that there are 6 molecules of carbon, 12 molecules of hydrogen and 6 molecules of oxygen in a glucose. Referring to the element periodic table, the corresponding atomic mass for C, H and O is 12, 1 and 16. So 1 $\mu$mol of glucose is around 0.18mg.

However, 100ml of tissue corresponds to different body weight from subject to subject. For example, the subject in [18] has 17kg fat over a total body weight 68kg, which means the fat tissue accounts for 17/68 (25%) of the body weight. According to the tissue density, 100ml of fat tissue is around 90g (approximately 106g for 100ml of muscle). So 100ml of fat tissue corresponds to 360g body weight. Substitute glucose into scaling, it can be assumed that in [18]:

$$1\mu \text{mol}/100\text{ml}/\text{min} = 0.5\text{mg/kg/min}$$

If some other subject has a fat percentage value of 40%. Then 100ml of fat tissue corresponds to 225g body weight. The scaling result will be different, which is:

$$1\mu \text{mol}/100\text{ml}/\text{min} = 0.8\text{mg/kg/min}$$

Those two examples show that if the distribution of adipose tissue vary a lot from subject to subject, the scaling result will be quite different from each other which is not ideal for our study.
Fortunately, all the subjects chosen (from Table 1) are healthy and have a similar BMI, which means the distribution of fat tissue and muscle tissue is close to each other (25% of fat and 35% of muscle).

For the reason of easy analysis and comparison, all the data are scaled by using the scaling fraction from Table 2.

Table 2. Scaling fractions for adipose and muscle

<table>
<thead>
<tr>
<th>Distribution in body</th>
<th>Amount of tissue (g/100ml)</th>
<th>Scaling fraction (mg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>38%</td>
<td>106g</td>
</tr>
<tr>
<td>Fat</td>
<td>25%</td>
<td>90g</td>
</tr>
</tbody>
</table>
3. Results

To make improvements on the old combination model, an initial test was done to prove that the combination model performs well enough with the previous assumptions. After the initial test, a series of comparisons were made from importing datasets from current publications [18] - [20]. Modifications then were implemented on the models to get a better fit with the datasets, such as reference to new theories in glucose distribution among organs, and redesign on part of the model to alter the dynamics of glucose uptake.

3.1 Initial test

The old combination model that has been given in Figure 1.5 is tested, and the simulation result is shown in Figure 3.1.

![Figure 3.1](Nyman2011 model: Glucose uptake in muscle tissue and adipose tissue)

**Figure 3.1.** Glucose uptake for muscle and adipose tissue from original combination model. Blue curve represents muscle tissue glucose uptake; black curve represents adipose tissue glucose uptake.

There are two curves in Figure 3.1, where the blue one is the simulation result for muscle tissue glucose uptake and the black one is the simulation result for adipose tissue glucose uptake. A meal is given at 0 min. The change of glucose uptake in both muscle and adipose tissue is reasonable to the respect of changing tendency. After the meal is given, due to the extra resource of glucose, the glucose uptake is increasing, and after reaching the peak, the curve starts to go down with the help of insulin and other glucose control mechanics.

The area under curve(AUC) is the amount of glucose uptake. Matlab function ‘trapz’ is used to calculate that AUC for both curves. AUC for fat tissue is 196 and AUC for muscle tissue is 751. This result meets the assumption made from previous researches, which is the amount of glucose uptake from muscle is 4 times than the glucose utilization by adipose tissue. Besides,
the glucose uptake speed is different between fat tissue and muscle, the peak time for adipose tissue glucose uptake curve is 90min which is shorter than that of muscle (98min). That peak time difference indicates fat tissue utilizes glucose faster than muscle.

In Figure 3.2, the simulation results from adipose tissue and muscle are added together which is the insulin-dependent glucose uptake according to the research [3]. Two thick red boundary curves are extracted from [3] which are the +/- standard deviation. The area within those two curves are the acceptable range. The simulation result is within the area between boundaries which means the simulation is correct with respect to [3].

**Figure 3.2.** Insulin-dependent (muscle and adipose tissue) glucose uptake comparison. Red curves are boundaries; blue curve is simulation result.

3.2 Compare original simulation with other papers

There are a few studies that have measured the dynamic glucose uptake in muscle and adipose tissues [18] - [20]. The datasets extracted from [18] - [20] will be used for comparison purpose in this section to test the assumptions made from the original model. The datasets will be classified as adipose tissue and muscle tissue and the comparisons between fat tissue and muscle will be made separately.

The datasets from [18] - [20] will be named Data1, Data2 and Data3 in the following figures. In Figure 3.3, the glucose uptake in adipose tissue in Data1 and Data2 have a similar changing trend. They start to increase after the meal is given at 0min and reach the peak at 30min and 40min separately. Also the AUC of those two curves are close, which are 49 and 56. However, the glucose uptake in adipose tissue in Data3 varies a lot from the other two, not only in changing tendency but also in amplitude. The reason for that variation is unknown, and I chose to remove Data3 from the following comparison.
A series of comparisons between datasets and simulations are given in Figure 3.4. The comparisons are made for muscle, adipose tissue, muscle and adipose tissue respectively. Meanwhile, the simulations are divided into Nyman2011 which is the original combination model; glucose uptake distribution modification simulation (M1); remote insulin dynamics modification simulation (M2); and blood flow modification simulation (M3).
Figure 3.4 Series of comparisons between simulations and modifications. ‘y’ axis in all figures represents glucose uptake. Four rows (1-4) are the original combination model and three different modifications that are applied to the mode: original combination model (Nyman2011), glucose distribution modification (M1), remote insulin dynamic modification (M2), blood flow modification (M3). Three columns (a-c) are for adipose tissue, muscle tissue, the sum of adipose and muscle tissue. Star curves are datasets from [18], [19]. Solid curves (muscle: pink, adipose: black, muscle + adipose: blue) indicate the simulation from original combination model (Nyman2011). Dotted curve means the simulation from glucose uptake distribution modification. Dashed-dotted curve is the simulation from insulin dynamic modification. Dashed curve indicates the simulation from blood flow modification.
The comparison between datasets and simulation on adipose tissue glucose uptake is given in Figure 3.4 1(a), where the star curves indicate datasets and solid curve indicates simulation from old combination model. It can be seen that model simulation has a larger AUC (190) than datasets (49 and 56), in addition, the peak time from our simulation is delayed. As can be seen from Figure 3.4 1(b), simulation for muscle glucose uptake has a bigger AUC (689) than datasets (335 and 328). Besides, the peak time in simulation is delayed.

The glucose uptake from muscle and adipose tissue are added together in Figure 3.4 1(c) to form the total insulin-dependent glucose uptake. The solid curve is the simulation, and the star curves are datasets. It can be seen that the curves of Data1 and Data2 have a lower AUC than the simulation (384 vs 929).

### 3.2.1 Summary on the comparison between the combination model and datasets

Both muscle tissue and adipose tissue from simulation have time delay problem and too high AUC. However, the datasets are in agreement with the previous assumption that the glucose uptake in muscle tissue is 4 time higher than the glucose uptake in adipose tissue. The reason for the higher AUC in our simulation might result from the wrong distribution of glucose uptake in adipose tissue and muscle tissue; and the time delay problem may be led by the incomplete dynamics of insulin and lack of new interactions. Corresponding modifications are made in the next section, also the simulation results are compared with the datasets again.

### 3.3 Modify the model to account for dynamic glucose uptake in different tissues

Two problems in our simulation are pointed out in last section, one of which is the peak time delay and another is the too high AUC. The solutions to those problems will be applied in sections 3.3 and 3.4 for adipose tissue and muscle tissue respectively.

#### 3.3.1 Add glucose uptake components

The assumption that is made in the original combination model is that glucose uptake in muscle is four times higher than in adipose tissue. The glucose uptake in muscle and adipose tissue is considered as insulin-dependent glucose uptake. The total glucose uptake has also an insulin-independent contribution from the central nervous system (Figure 3.5).

![Glucose uptake diagram](image)

**Figure 3.5.** Assumption in original combination model. Glucose is utilized by two parts, one is insulin-dependent part and one is insulin-independent part; insulin-dependent part contains muscle and adipose tissue, where the muscle tissue utilizes four times more glucose than the adipose tissue.
However, that assumption is challenged by the difference between model simulations and the new datasets. Going through other researches, there are a few different assumptions about the glucose uptake distribution among tissues [28], [29]. The reason for making assumptions instead of measuring is due to the difficulty of measurements. For example, the glucose uptake in liver. So far there hasn’t been a direct method for measuring the glucose uptake in liver due to the complexity in physiological structure. Besides, when measuring the glucose uptake for muscle and adipose tissue, the position of catheters will also alter the final results. Those uncertain and hard-control factors limit the measurements of glucose uptake in experiments, as a compensation, assumptions are made to have a brief idea for those regulations in the body.

Two assumptions for glucose uptake are given in Figure 3.6(a) and 3.6(b) respectively. In Figure 3.6(a), the total amount of glucose uptake is divided into three parts. One third of glucose goes to the muscle and fat tissue; one third of glucose goes to liver and the rest goes to central nervous system (CNS) and red blood cells (RBC). In Figure 3.6(b), it shows the disposal by several tissues and organs of a 100g glucose meal. Similarly, to Figure 3.6(a), one third of the glucose goes to liver and one third goes to muscle and adipose tissue. However, part of the rest glucose goes back to liver while the other is used by brain and kidney.

(a)

(b)

**Figure 3.6 (a).** New assumption for glucose uptake distribution [28], 33% of glucose goes to muscle and fat tissue; 34% of glucose goes to liver; 33% of glucose goes to central nervous system and red blood cells. (b). Among 100g of glucose, 45g is utilized by liver, 27g by muscle, 5g by adipose tissue, 8g by kidney and 15g by brain.
From Figure 3.6, it is clear that the components in our original combination model (muscle tissue, adipose tissue and central nervous system) are not enough to describe the total glucose uptake and that explains the too high AUC from the previous simulations. One solution to solve that problem is decreasing the portions of muscle tissue and adipose tissue while still keeping their relationship (glucose uptake from muscle is 4 times higher from adipose tissue). New distribution is shown in Figure 3.7.

Figure 3.7. New distribution of glucose uptake. Glucose is utilized by two parts: fat, muscle and liver belongs to the same group where the percentages of glucose of each component are 10%, 40% and 50%. Central nervous system is another part which keeps a steady use of glucose.

3.3.2 Comparing simulation with datasets

The muscle glucose uptake in the original combination model is given by Equation (3.1). As mentioned at the end of last section, I want to decrease the portion of muscle glucose uptake to a half.

\[ U_m = \frac{V_{\text{mm}ax} \cdot G_t}{k_m + G_t} \] (3.1)

where \( V_{\text{mm}ax} \) (3.2) is the parameter that depends on remote insulin, \( G_t \) is the glucose in tissue, \( k_m \) is a constant.

\[ V_{\text{mm}ax} = \text{portion} \cdot (V_m + V_x \cdot \text{INS}) \] (3.2)

The modification is to reduce the ‘portion’ from 0.8 to 0.4.

In original combination model, adipose tissue glucose uptake is written as Equation (3.3):

\[ U_f = C_1 \cdot \left( C_2 \cdot \text{GLUT}4m \cdot G_t + C_3 \cdot \text{glut}1 \cdot G_t + C_4 \cdot (\text{INS} + INS_b) \right) \] (3.3)

where \( C_1, C_2, C_3, C_4 \) are scaling parameters, \( \text{GLUT}4m \) is an insulin dependent term. INS is the interstitial fluid insulin and the subscript b means basal value. In this section, (3.3) is divided by two. The results are given in Figure 3.4 2(a) – 2(c)
For muscle tissue, in Figure 3.4 2(b), the AUC for the simulation is 358 which is very close to
the datasets (335 and 328), which means the new assumption works well for the muscle tissue.
For adipose tissue, in Figure 3.4 2(a), AUCs for simulation and datasets are 89, 49 and 56
respectively, the higher part of the simulation can be caused by the slower decrease after the
peak point. In order to make the simulation closer to the datasets, changes on dynamic terms
will be done later. In Figure 3.4 2(c), AUCs for simulation and datasets are 473 and 384,
which is an improvement comparing to the result in 1(c). However, the simulation and datasets fit at
the beginning before reaching the peak while our simulation has a slower decreasing speed after
the peak. In addition, the time delay problem still exists. In the next section, the time delay
problem will be discussed and modification on remote insulin will be used to try to solve the
delay problem.

3.4 Change dynamics of remote insulin

In the last section, glucose uptake distribution from different tissues has been improved with
decreasing the portion of adipose tissue and muscle into half. The simulation result for muscle
is good in quantity while that of adipose tissue is not ideal both in quantity and dynamics.
Besides, changing the glucose uptake distribution will not alter the dynamic of glucose uptake
which means the time delay problem mentioned before still exists.

From Equation (3.2) and (3.3), it can be found that the remote insulin is one variable that can
control the dynamics of muscle and adipose tissue glucose uptake. So in this section,
modifications will be applied to remote insulin to see the corresponding effects on both adipose
tissue and muscle glucose uptake. In the original combination model, remote insulin is denoted
as Equation (3.4).

\[ I\dot{NS} = -p \cdot INS + p \cdot (I - I_b) \]  \hspace{1cm} (3.4)

where INS is the interstitial fluid insulin, I is the plasma insulin, subscript b means basal value
and p is a scaling parameter.

One idea to change the dynamics of INS is to give different values to the scaling parameter ‘p’.
Meanwhile, since adipose tissue and muscle tissue vary a lot from each other, different remote
insulin should be applied to them which means there should be two remote insulin terms,
\( INS_{\text{muscle}} \) and \( INS_{\text{adipose}} \).

After trying several sets of values, the final remote insulin for muscle and adipose tissue have
been decided as Equation (3.5), (3.6).

\[ INS_{\text{muscle}} = -1.65 \cdot INS_{\text{muscle}} + 1.3 \cdot (I - I_b) \]  \hspace{1cm} (3.5)

\[ INS_{\text{adipose}} = -1.25 \cdot INS_{\text{adipose}} + 0.6 \cdot (I - I_b) \]  \hspace{1cm} (3.6)

The results after insulin dynamic modification are given in Figure 3.4 3(a) – 3(c). 3(b) is the
result for muscle tissue, the AUC of muscle after apply insulin dynamic modification is (315)
which shows a decrease comparing to the simulation (358) in 2(b) and also closer to datasets
(335 and 328). The peak time is also earlier than 2(b). For adipose tissue, in 2(a) there is an
obvious decrease in AUC and also the peak time with new remote insulin is (70min) comparing
to the old simulation (90min). However, the decreasing speed after the peak from our simulation is still slower than datasets.

After the insulin dynamic modification, the result of the insulin-dependent part (muscle and adipose tissue) is given in 3(c), it can be seen that both AUC and peak time are improved comparing to 1(c) and 2(c).

In the previous sections, both glucose uptake distribution among different tissues and dynamic of remote insulin are modified. It is assumed that liver takes the same amount of glucose as the sum of adipose tissue and muscle, though the dynamic of liver glucose uptake take is unknown. The remote insulin is changed to make the glucose uptake fit better with the datasets dynamically. After all the improvements, the glucose uptake in the modified model (liver, muscle, adipose tissue and central nervous system) are added together and compared with the original combination model. The comparison is shown in Figure 3.8. The new model after modifications behaves good, since the result is still between the boundaries, while the dynamics of the new model is closer to the datasets.

**Figure 3.8** Comparison between original combination model (Nyman2011) and modified model. Read curves are the boundaries which set the acceptable range for the simulation. Solid blue curve is the result from original combination model (Nyman2011); the dotted-dashed curve is the result from the modified model.
3.5 Blood flow effect on glucose uptake

A possible explanation to the rapid decrease in glucose uptake in the adipose tissue after a meal is the blood flow that transport the glucose throughout the body. The effect from blood flow on glucose uptake have been studied in [30]. In that study, bradykinin which can dilate the vessel to increase the blood flow, was infused intra-arterially for 50mins before the measurement of blood flow. One conclusion from [30] is that the glucose uptake in adipose tissue will not vary much when there is no change in insulin. However, after taking a meal, insulin will alter with time. In order to use the knowledge from this blood flow effect study, both insulin and blood flow have to be taken into account.

In the current combination model, the adipose tissue glucose uptake is written as Equation (3.3), where the last term $C_s \cdot \left(INS + INS_b\right)$ was considered as blood flow effect. This term was modified to compare with data in [30]. First, a new term ‘blood flow’ was created for adipose tissue (Equation 3.7) and fitted with corresponding dataset which is given in Figure 3.9.

$$bf = \left(bf_b + C_s \cdot \left(INS + INS_b\right)\right) \cdot BRF \quad (3.7)$$

![Figure 3.9. Adipose tissue blood flow comparison. The dotted-dashed curve is the simulation; solid curve is dataset.](image)

where ‘bf’ stands for blood flow, and the subscript ‘b’ means basal value, $C_s$ is the scaling parameter and BRF represents the bradykinin fraction. When there is no bradykinin, BRF will be given the value 1 which means there’s no change in blood flow. The new blood flow term fits well with the dataset. After that a term for blood flow effect is designed as Equation (3.8).

$$bfe = \left(bf - bf_b\right) \cdot \left(INS - INS_b\right) \cdot C_6 \quad (3.8)$$

this blood flow effect term meets the conclusion from the previous study [30], which is when there is no change in insulin, the blood flow should have no effect on the adipose tissue glucose uptake. The new version of adipose tissue glucose uptake is given in Equation (3.9).
\[ U_t = C_1 \cdot (C_2 \cdot GLUT4 \cdot C_t + C_3 \cdot \text{glut1} \cdot C_t + \text{bfe}) \] (3.9)

A comparison of adipose tissue glucose uptake after blood flow modification is given in Figure 3.4 4(a), it can be seen that the peak time improves slightly comparing to 2(a) and 3(a), however, the simulation curve doesn’t go down as fast as the data. That difference in descending leads to the variance in AUC, where the simulation is 95 comparing to datasets (49 and 56).
4. Discussion

After several modifications and improvements to the original combination model, now the new combination model for multi-level glucose uptake in muscle and adipose tissue is able to provide us a better fit with datasets (Figure 3.4). The first improvement of the model is a changed distribution of glucose uptake in different tissues. The glucose uptake has been decreased into half for both muscle and adipose tissue, which agrees with new previously not used data (Figure 3.7). The main reason for the previous discrepancy was the lack of glucose uptake by the liver. The second improvement is a modification of the dynamics of remote insulin in order to influence the regulation of both muscle and adipose tissue glucose uptake. These improvements were enough to for a good agreement with muscle glucose uptake, while for adipose tissue glucose uptake still the simulation does not fit with the datasets. Therefore, in a third attempt, a study on the effect of blood flow on adipose tissue glucose uptake is used to expand the model with a blood flow effect. With these results in mind, let us now turn to a brief summary of the pros and cons of the model.

4.1 Strength and weakness of this project

The results from this thesis show that the new combination model performs better than the original combination model [15] with comparison to datasets [18], [19] both in quantity and dynamics. The current combination model contains a new theory in glucose distribution [28], [29] which adds liver as one of the glucose uptake organ to explain the higher glucose uptake amount in the original merging model. What is more, interstitial fluid insulin has been modified to adjust the glucose uptake rate in both adipose tissue and muscle. One other improvement is the re-design of ‘blood flow effect’ term, which makes the simulation of adipose tissue glucose uptake fit better with other datasets. Apart from a better accuracy, the new model keeps the most part of the original combination model which means only a few but necessary changes are made. That makes it easier for researchers to compare the new model with the original one and to make further improvements.

However, there are still some shortcomings of this new combination model. First, the amount of available data that the model can be built upon is limited. Only a few available papers contain glucose uptake from different tissues (muscle & adipose tissue) in response to a meal intake, which narrows the number of comparable samples. For example, in Figure 3.3, the curve for data3 is removed due to its variance (both amplitude and dynamics) from the other datasets. A second shortcoming is the limited knowledge on the glucose uptake distribution after a meal in humans. In this project, I simply assume that liver takes the same amount of glucose as the sum of fat and muscle tissue. This assumption might be an underestimation of the liver glucose uptake, and I have found no measurements on liver glucose uptake in humans. Therefore, also the dynamics of the liver glucose uptake is unknown, which affect the dynamics of the total glucose uptake. The most important shortcoming is the descending speed of glucose uptake after peak value in adipose tissue. Differing from all the datasets, our model has a much slower decreasing velocity while the ascending rate before the peak fits well with data. One assumption for that shortcoming is glucose-6-phosphate (G6P) should be taken into account for the adipose tissue glucose uptake. Since the intracellular activity of G6P affects so many variables such as intracellular glucose concentration and the out flux of glucose from the cell.
4.2 Future work and outlook

Part of the future work is to continuously improve the model by solving the limitations from last section. Since the muscle part is acceptable in the modified combination models, more attention will be paid to the adipose tissue glucose uptake. Also, the dynamic of liver glucose uptake needs further research both theoretically and experimentally.

The function of this multi-level multi-scale model is not limited in simply simulating glucose dynamics, it can also be used to study the effects from both meals and drugs. The meals input used in this project is a mixed meal with normal amount of different nutrients, in the future, different types of meals such as high-calorie food can be studied with corresponding metabolites regulations. Similarly, the effects of drugs on internal dynamics can also be studies for estimating and testing purpose by combing with the model.
5. Conclusion

The multi-level and multi-scale model developed herein can simulate glucose utilization both at the whole-body and cellular level. In this thesis, an existing model has been improved in several steps. A few important findings are listed below:

1. The original combination model cannot explain the dynamic tissue-specific glucose uptake measurements
2. With a new glucose distribution where 40% of glucose is utilized by muscle, 10% by adipose tissue, 50% by liver, the model is in better agreement with all available data.
3. With a new tissue-specific remote insulin, the glucose uptake for muscle and adipose tissue fit better with datasets comparing to the original combination model.
4. With a new blood flow equation, the model now is able to simulate the blood flow effect correctly under different circumstances of insulin.

All in all, the model improvements and the resulting model provide a deeper understanding into glucose regulation in humans, and this leads us one step closer to reliable model-based end-usage in drug development, research, and medical diagnosis.
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