CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Formal Modeling and Analysis of Insulin Resistance Pathway

by

Alamdar Hussain

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2020

I would like to dedicate this thesis to my Family, Friends and Teachers

CERTIFICATE OF APPROVAL

Formal Modeling and Analysis of Insulin Resistance Pathway

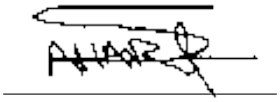
by

Alamdar Hussain

(MBI173005)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Abdur Rehman	NUST, Islamabad
(b)	Internal Examiner	Dr. M.Tanvir Afzal	CUST, Islamabad
(c)	Supervisor	Dr. Sahar Fazal	CUST, Islamabad



Dr Sahar Fazal
Thesis Supervisor
May, 2020

Dr. Sahar Fazal
Head
Dept. of Bioinformatics and Biosciences
May, 2020

Dr. Muhammad Abdul Qadir
Dean
Faculty of Health and Life Sciences
May, 2020

Author's Declaration

I, **Alamdar Hussain** hereby state that my MS thesis titled "**Formal Modeling and Analysis of Insulin Resistance Pathway**" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

Alamdar Hussain

Registration No:MBI173005

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Formal Modeling and Analysis of Insulin Resistance Pathway**" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been dully acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

Alamdar Hussain

Registration No:MBI173005

Acknowledgements

First of all, Thanks to Almighty Allah for blessing me with abilities to carry out all of my research related activities. A lot of people helped and assisted during my research project and in the compilation of this document. I am grateful to all of them, and wish them the best in their endeavours. However, there are some, the contributions of whom make them worthy of a thanking by name. I will start with Ahtisham Fazeel, an RCMS MS scholar who, as Allah would have it, had been my batch mate during BS in CUST. I thank him for the time he spent discussing theories with me, and assisting in working out different angles to each obstacle. Of course, the contributions of one Mr. Iftikhar sheikh cannot be ignored even if wanted to. I thank my supervisor, Dr. Sahar Fazal, who, unlike those taking academics as 9-to-5 desk job, is a true mentor, ever vigilant, always pushing me to the edge so that I can learn, to give my best, and most importantly to think creatively and out-of-the-box. This research and this document would not have been completed without her.

Alamdar Hussain

Registration No:MBI173005

Abstract

The transformation in the maintenance of glucagon via disturbance in insulin is considered a crucial cycle leading to diabetes. Mainly, the insulin resistance pathway is considered as the common reason for the improper working of insulin. Different drugs have been identified to deal with diabetes but all of them are insulin booster and some of them are to decrease the amount of sugar to absorb in stomach, which drives the need for new drug targets. Identifying a drug target is cost and time consuming task via classical drug identifying techniques because of the overhead of different parameters involved in the process. We utilize a Qualitative modeling framework which neglects the overhead of continuous kinetic parameters usually identified via lab experiments that are time and cost consuming. Furthermore, techniques like process hitting, model checking, hybrid modeling, and Petri nets modeling are utilized to investigate the dynamics of the system for the identification of new drug targets on a cellular level. All of the analyses from this study shows that Insulin Receptor is degraded earlier then Insulin Receptor Substrate and AKT, and PTPS is activated earlier then other inhibitors, also only inhibitor that inhibits Insulin Receptor is PTPS which shows that while dealing with diabetes in case of insulin resistance in muscles PTPS side by side with obesity acts as a modulator to take the system into the diseased state.

Contents

List of Figures

List of Tables

Abbreviations

AKT	Protein Kinase
AS160	Akt Substrate
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
ALGU	RNA Polymerase Sigma-H Factor
Bio-LHA	Biological Linear Hybrid Automata
BRN	Biological Regulatory Network
CTL	Computational Tree Logic
GLUT4	Insulin-Regulated Glucose Transporter
GRN	Gene Regulatory Network
INS	Insulin Receptor
IRS	Insulin Receptor Substrate
LAR	Antigen Related Phosphate
ODE	Ordinary Differential Eqvation
PI3K	Phosphoinositide 3-Kinases
PTPS	Protein Tyrosine Phosphatases
TNF-'*alpha*'	Tumor Necrosis Factor Aplha

Chapter 1

Introduction

Human body needs calories to perform tasks and that energy is present in food which contains energic components such as; sugar, fats and proteins and non-energic components including vitamins, minerals and water. Carbohydrates are the main source of energy to the body and brain; carbohydrates are basically sugar and starch having direct impact on the level of sugar in blood which shows its importance to be monitored properly and regularly. The digestion of carbohydrates is also very fast. A healthy life needs a proper life style which includes daily exercise to burn calories. Lack of exercise lead to many problems including obesity, caused by consuming too much food and moving little, is one of the major among them. If a person is taking too much energy from its diet but do not use that energy in the form of exercise or other physical activity it will ultimately convert into fats. Diabetes is also a persistent disease occurring due to the pancreas when it doesn't create sufficient insulin or even body can't successfully make use of the insulin that was created.

Type two diabetes mellitus (T2DM) is really a main open public health condition globally. The actual occurrence of this raise within adults, lack of exercise, diet plan, being overweight, hereditary, reduced sugar threshold; and also the frequency rate associated with diabetes is actually growing within kids [106,107]. There are lots of genetics adding to this particular issue, from that thirty six genes

are known and 10 percent associated with hereditary can be explained [108].Figure 1.2 shows The induction associated with reactive oxygen (RO) generation as well as inflamation (NF-kB activation) through macronutrient consumption, being overweight, fatty acids, leptin, an infection, cigarette smoking, psychological tension as well as hereditary elements. Hereditary elements may actually lead as much as 30-70 percent instances associated with T2DM [109,110]. The T2DM is actually polygenic as well as heterogenous, therefore several genes are participating, and various combinations of genes are likely involved within T2DM.

Insulin is a hormone which adjusts blood sugar levels. Hyperglycaemia, or increase blood sugar levels, is common effect associated with uncontrolled diabetes as well as with results in severe harm to most of the body systems, particularly the nerves as well as blood vessels (WHO,2018). The actual worldwide frequency associated with diabetes within grown-ups may be growing more than current years. Within 1964, it had been believed that thirty million individuals experienced diabetes [1]. Less than 45 decades afterwards, the particular WHO projected a report demonstrating 171 thousand humans as diabetic [2]. International Diabetes Federation (IDF) believed the actual worldwide frequency will be 151 million within 2000, in 2003 194 million, in 2006, 246 million, 285 million in 2009, in 2011 about 366 million and 382 million in 2013 [3][4][5][6][7][8].

Even though classification associated with diabetes into type 1 and type 2 is essential and is very useful for treatment methods [54]. The conventional classification of diabetes is proposed by American Diabetes Association in 1997 as type 1, type 2 and other types. The difference between type 1 and type 2, diabetes depends on the genotype as well as being overweight and for that reason insulin resistance is actually the center of the theory [55]. The rise within diabetes happened in every nation around the world, and also inside villages along with towns. Precise worldwide, local, country-level estimations as well as projections associated with diabetes frequency are essential with regard to avoidance as well as treatment method to be planned and in addition to be monitored [9]. All living

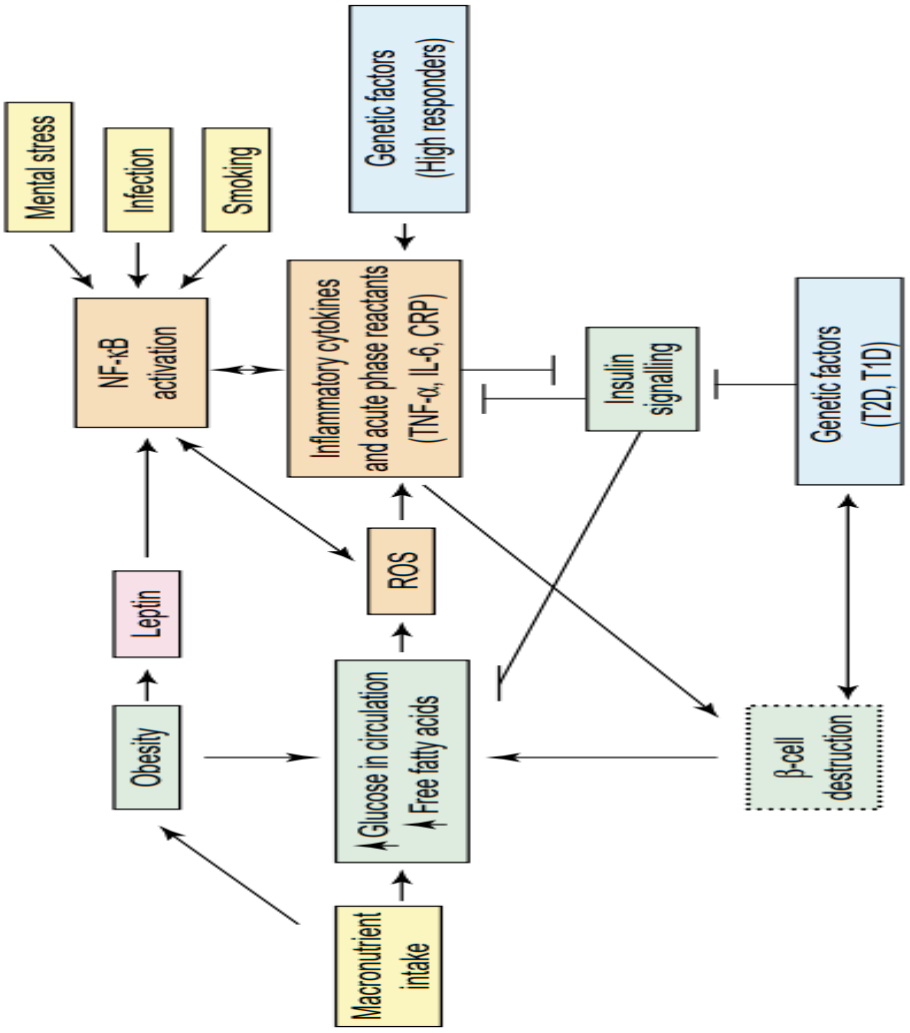


FIGURE 1.1: The induction association

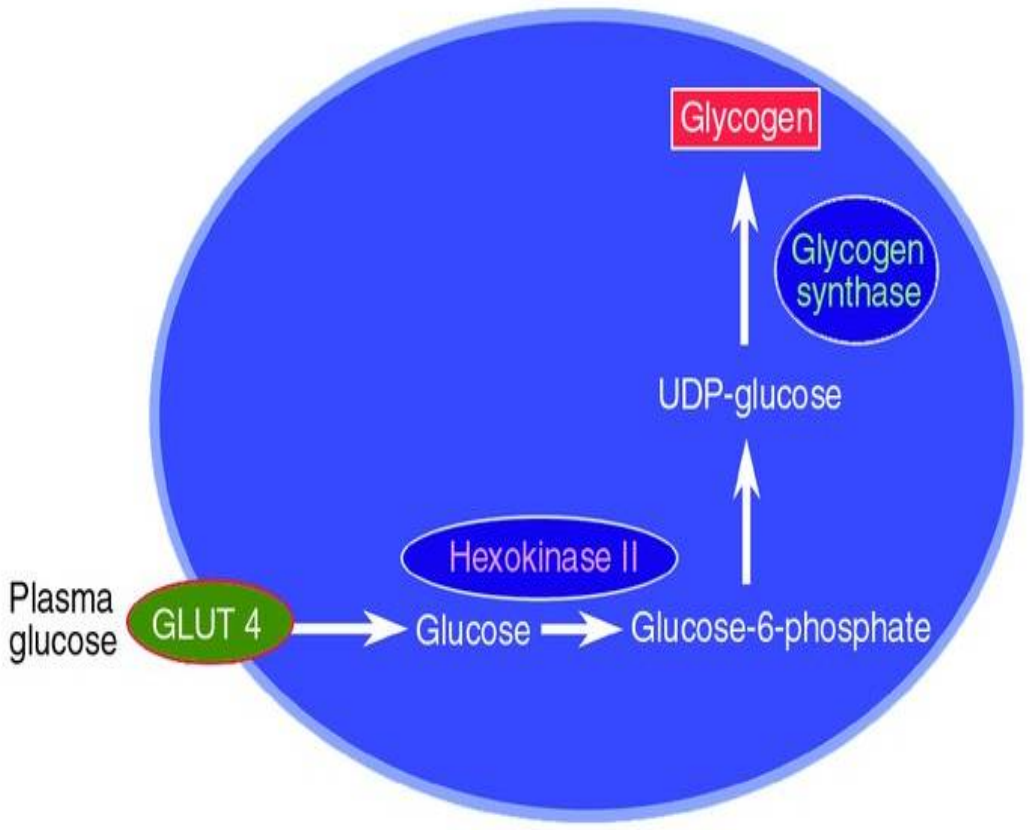


FIGURE 1.2: Cellular mechanisms in insulin resistance. Adapted from [10.1172/JCI10583]

organisms have biological pathway that contains different entities that communicate with each other and make a process go in a highly efficient manner. These entities can provoke a new pathway or gene expression and vice versa. These efficient systems can also have problems, a little change can disturb whole pathway and can lead to a disorder that can be death causing. To check these biological pathways outside the living organisms we have interdisciplinary field known as systems biology focusing on computational and mathematical modeling of complex biological system.

1.1 Modelling of Biological Systems

In the era of advance science new sequences and genomes are discovered performing a particular work in the form of communication which makes a pathway to perform homeostasis in normal functions where genes or proteins are represented by nodes and their interaction whether they are activating or inhibiting one another are represented by edges. To study these pathways there are many strategies, with the help of computational approaches Quantitative modeling based on differential equations and Qualitative modeling based on validation where formal modeling plays an important job. To deal with the nonlinear behavior of the biological systems Thomas and Kauffman introduced a qualitative modeling approach. The actual current function in the region associated with computational techniques the field of biology significantly concentrates about the improvement associated with techniques as well as resources to comprehend the performance of those systems to recognize possible medication focus on associated with complicated disease.

1.2 Purpose

The study focuses on using the informatics to identify therapeutic target that can marshal towards treatment of diabetes.

1.3 Problem Statement

The change within normal life style as well as nutritional intake, the prevalence associated with office work and sedentary life has led to high blood glucose level in people. A vital factor is the cell ignoring the insulin in the body, basically resisting the insulin discharged in the body leading to Insulin resistance resulting in Diabetes mellitus a chronic metabolic disorder. Analysis of dynamics in insulin concentration in blood is of vital importance in detailed understanding of impact

of insulin. In vitro experiments tend to be slow, cost consuming and are often infeasible in analysis of such metabolic disorders. Insilico computational executable models of biological systems can be used to analyze the role of biological regulatory network in insulin for predictions, preparation and elimination of cost and time bar. Moreover, identifying a specific drug target is a rigorous, extravagant and time consuming task via classical drug identifying methods because of the overhead of different parameters involved in the process. Application of modeling such as qualitative framework can reduce the overhead of identifying the kinetic parameters and in identification of drug target that can be further utilized in further insilico studies as a treatment strategy against diabetes.

1.4 Aims and Objectives

- To identify entities (Protein) tend to be triggered step by step using formal modeling.
- To identify impacts of activation as well as degradations of entities on the pathway by computing delay constraints for important trajectories.
- Model and simulate the Insulin Resistance Pathway with Stochastic-PetriNet modeling to confirm the outcomes of hybrid modeling.

Chapter 2

Literature Review

The actual elevated frequency associated with being overweight offers concentrated interest on the globally issue that isn't among starvation or even an infection. In America, just about one third regarding adults are normal in weight [76], as well as comparable more are now being reported overweight globally [77]. Being overweight is actually related to a number of problems, probably the most damaging associated with which can be type 2 diabetes. In the change of the hundred years 171 million people had been believed to possess diabetes, which is actually likely to be more in order to 366 million in 2030 [78]. The obesity along with type two diabetes are generally linked to insulin resistance [79] However the majority of overweight, insulin-resistant people don't have hyperglycaemia. In normal condition, the pancreatic islet /*beta*/ -cells improve insulin discharge adequately in order to conquer the actual decreased effectiveness associated with insulin, therefore sustaining regular sugar threshold [80, 81]

With regard to being overweight as well as insulin resistance to become related to type two diabetes, /*beta*/ -cells should struggle with regard to reduced insulin sensitivity. The disturbance in /*beta*/ -cell function is also present within people who are from high-risk associated with diabetes even if their own blood sugar levels continue to be regular [82].Non-esterified fatty acids (NEFAs) stimulate insulin resistance as well as hinder /*beta*/ -cell function, indicating all of them

the most likely reason

2.1 Insulin

Insulin is a peptide hormone discharged by the /*beta*/ cells of the pancreatic islets of Langerhans and keeps up ordinary blood glucose levels by encouraging cell to uptake glucose, managing sugar, lipid and protein digestion and advancing cell division and development through its mitogenic impacts [11]. In 1889, German researchers Minkowski and von Mering discovered from their trial work with animals that pancreatectomy prompted the enhancement of extreme diabetes. They guessed that a substance discharged by the pancreas was in charge of metabolic control. In January 1922, the principal human examinations started on a 14 year old diabetic kid whose clinical symptoms and biochemical variations from the normal were basically turned around [12]. It contains A and B chains individually, connected by disulphide connects and containing 51 amino acids with molecular weight of 5802 g/mol [13].

2.2 Insulin Receptor Signaling

Insulin signaling entails the cascade associated with occasions started through insulin joining in order to it's cellular area receptor, initiating receptor autophosphorylation, as well as activation associated with receptor tyrosine kinases, leading to tyrosine phosphorylation of insulin receptor substrates (IRSs) such as IRS1, IRS2, IRS3, IRS4, Gab1, and Shc [101,102]

Joining associated with IRSs towards the regulating subunit associated with phosphoinositide 3-kinase (PI3K) by Src homology 2 (SH2) domains leads to activation of PI3K, that phosphorylates membrane phospholipids as well as phosphatidylinositol 4, 5-bisphosphate (PIP2) on the 3' position. This particular complex triggers the actual 3-phosphoinositidedependent proteins kinases (PDK-1

as well as PDK-2) leading to activation of Akt/protein kinase (PKB) as well as atypical proteins kinase C (PKC), all of that are serine/threonine kinases [103,104] as shown in fig 2.1.

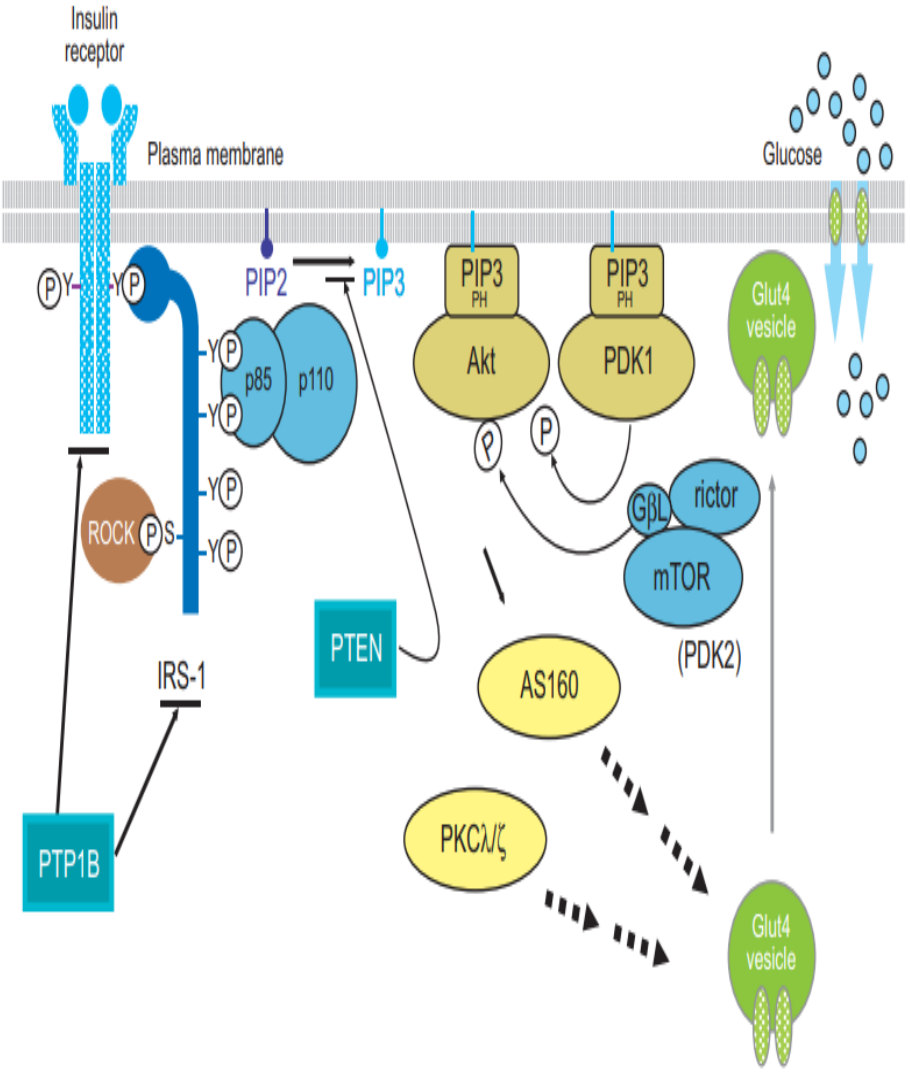


FIGURE 2.1: Diagrammatic representation of Insulin receptor signaling

2.3 Obesity

The actual frequency associated with being overweight in our midst grown ups elevated to 20. 9 percent within 2001 that was 19. 8 percent within 2000, a

rise of 5. 6 percent. From 1991 the actual portion of those that have been overweight increased by 74 percent (1991 frequency, 12 percent). That frequency is approximately 21.4 million overweight males as well as 22. 9 million overweight females, and overall 44. 3 million overweight all in US adults. The actual portion associated with adults having a BMI of 40 or more had been 2.3 percent (1.7 percent males, 2.8 percent women) vs 2.1 percent within 2000 as well as 0.9 percent within 1991. Amongst racial, blacks experienced the greatest number associated with being overweight (31.1 percent). Among states, Mississippi had the highest number associated with being overweight (25. 9 percent) as well as Colorado the lowest (14.4 percent). From 1991, the actual portion associated with obese adults elevated from 45 percent in order to 58 percent of these obese within 2001, 65. 9 percent were males as well as 49. 9 percent were females.

The actual frequency of those having diabetes elevated in order to 7. 9 percent within 2001 from 7. 3 percent within 2000, a rise associated with 8. 2 percent as well as a rise associated with 61 percent from 1990 (4.9 percent). Therefore, within 2001, approximately 16. 7 million all from US adults might have been identified of having diabetes (6. 9 million males; 9. 8 million women). Within 2001, 3.4 percent adults (2.9 percent males, 3. 8 percent women) had been both overweight as well as had diabetes, a rise of just 1. 4 percent from 1991. Blacks experienced the highest rate of diabetes (11. 2 percent) amongst all, as well as adults with less then higher college education experienced the greatest (13. 0 percent). People older then 60 years, 15. 1 percent had diabetes. The state of Alabama experienced the highest rate associated with diabetes (10. 5 percent) as well as Minnesota had (5. 0 percent)[105] Fig 2.2 shows the Prevalence of Obesity and Diagnosed Diabetes Among US Adults, 1991 and 2001.

2.4 Diabetes

Diabetes mellitus is abnormal amounts of sugar (glucose) in the blood. This might be on the grounds that insulin isn't being delivered by any means or not made

at adequate dimensions, or not as powerful as it ought to be. The most widely recognized types of diabetes are type 1 diabetes, which is an immune system issue, and type 2 diabetes, which is also related with overweight. Gestational diabetes is a type of diabetes that happens in pregnancy, and different types of diabetes are exceptionally uncommon and are brought about by a mutation. Type 2 diabetes generally happens in grown-ups who are overweight.

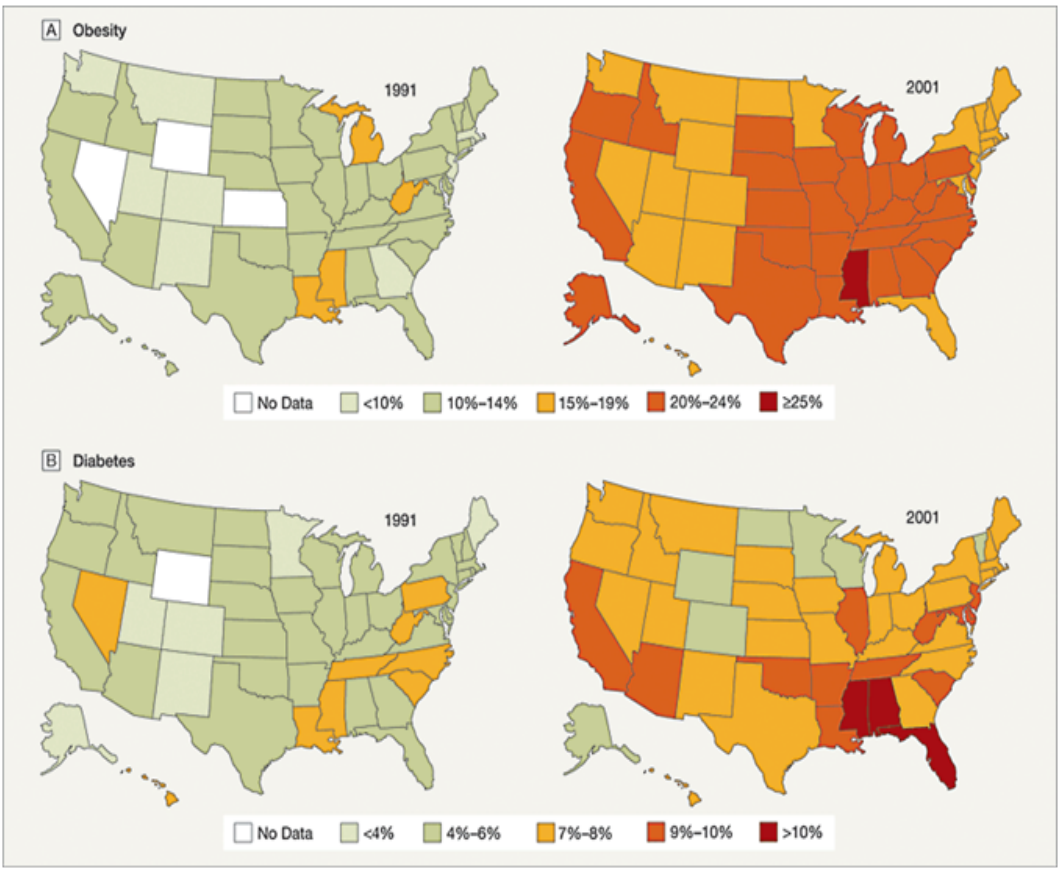


FIGURE 2.2: Prevalence of Obesity and Diagnosed Diabetes Among US Adults, 1991 and 2001

There are numerous basic factors that add to the high blood glucose levels in these people. A vital factor is the cell ignoring the insulin in the body, basically disregarding its insulin discharges. A second factor is the falling creation of insulin by the beta cells of the pancreas. Accordingly, a person with type 2 diabetes may have a blend of inadequate discharge and insufficient activity of insulin [14]. Doctors have watched the impacts of diabetes for many of years. One of the impacts of diabetes is the presence of glucose in the urine. Old Hindu

works record how flies and other ants were attached towards the waste of diabetics person.

The Indian doctors explained the sweet taste of waste of diabetics person, and for a long time to come, the sweet taste of waste of diabetics person was key to finding. The total term "diabetes mellitus" came into existence in 1674 by Thomas Willis, personal doctor to King Charles II [15]. The actual elevated occurrence associated with type 2 diabetes within youngsters is principally because of the alter within the way of life from the kids when it comes to much more inactive existence as well as much less healthy food choices. Being overweight may be the main cause of insulin opposition that is primarily accountable for type 2 diabetes. [16].

2.5 Insulin Resistance and Obesity

Variances within insulin sensitivity happen throughout the regular existence period, along with insulin resistance becoming noticed throughout puberty [83] as well as pregnancy [84], with aging [85]. On the other hand way of life variation for example exercises as well as high number of carbohydrates consumption tends to be related to high insulin resistance [86, 87, 88]. Probably the most crucial emergence associated with metabolic disease is actually being overweight. Adipose cells modulates metabolic process through releasing NEFAs as well as glycerol, the body's hormones — such as leptin and adiponectin and proinflammatory cytokines [89,90,91]. Retinol-binding protein-4 (RBP4) induces insulin resistance via reduce phosphatidylinositol-3-OH kinase (PI(3)K) signalling within muscle mass as well as improved expression of the gluconeogenic enzyme phosphoenolpyruvate-carboxykinase within the liver via a retinol-dependent mechanism [92]. By comparison, adiponectin functions being an insulin sensitizer, stimulating fatty acid oxidation within an AMP-activated proteins kinase (AMPK) as well as peroxisome proliferator triggered receptor/*alpha*/ (PPAR-/*alpha*/) dependent manner [93].

Along with adipocyte-derived factors, elevated discharge of tumor necrosis factor-/*alpha*/ (TNF-/*alpha*/), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and extra items associated with macrophages along with other cells which populate adipose cells may also possess a part within the developmentt associated with insulin resistance [94].

TNF-/*alpha*/ as well as IL-6 act via classical receptor-mediated processes in order to promote both c-Jun aminoterminal kinase (JNK) and also the IK/*beta*/ kinase-/*beta*/ (IKK-/*beta*/)/nuclear factor-K/*beta*/ (NF-K/*beta*/) pathways, leading to up regulation associated with possible mediators associated with inflammation which can result in insulin resistance.

The actual discharge associated with NEFAs could be the most important element in modulating insulin sensitivity. Elevated NEFA amounts tend to be seen in being overweight as well as type two diabetes, and therefore are linked to the insulin resistance in both [95,96].

Insulin resistance evolves in several hours of increase within plasma NEFA within humans [97]. On the other hand, insulinmediated glucose uptake as well as its tolerance enhance by reduction in NEFA using the antilipolytic agent acipimox [98]. Increased in intracellular NEFAs may lead to competition along with glucose with regard to substrate oxidation resulting in the inhibition associated with pyruvate dehydrogenase, phosphofructokinase as well as hexokinase II activity [99].

It's been proposed that elevated NEFA delivery or even reduced intracellular metabolic process associated with fatty acids leads to a rise within the intracellular content associated with fatty acid metabolites for example diacylglycerol (DAG), fatty acyl-coenzyme the (fatty acyl-CoA), as well as ceramides, that, trigger the serine/threonine kinase cascade resulting in serine/threonine phosphorylation associated with insulin receptor substrate-1 (IRS1) as well as insulin receptor substrate-2 (IRS-2), along with a decreased capability of those substances in order to trigger PI(3)K [100]. Eventually, activities downstream insulin-receptor signalling are usually decreased.

2.6 Mechanisms of Insulin Resistance

Insulin resistance is actually extensively understood to be the actual decrease in insulin capability to promote sugar uptake through entire body peripheral tissue. At normal, insulin triggers sugar uptake through provoking the actual canonical IRS-PI3K-Akt path as well as through phosphorylation and inactivating Akt substrate one hundred sixty (AS160), the proteins which, whenever triggered, helps prevent sugar transporter (GLUT)4 translocation towards the membrane layer. Therefore, through suppressing AS160, insulin encourages the actual GLUT4 translocation through internal vesicules, promoting fusion towards the plasma membrane layer and therefore sugar uptake [40]. Data suggests that faty acid are usually significantly improved inside obesity and also associated-diseases, may play a role inside the advancement regarding skeletal muscle insulin resistance [41, 42]. With this sense, extended exposure associated with skeletal muscle as well as myocytes in order to higher amount of fatty acids results in serious insulin resistance [43, 44].

Suggested theory was, increased fatty acid oxidation boosts the actual manufacturing acetyl-CoA leading to inhibition associated with pyruvate dehydrogenase function as well as increased amount of citrate in the tricarboxylic acid cycle. Citrate and increased ATP/ADP ratio slow up the functionality associated with phosphofructokinase and therefore sugar flux with the glycolytic path, leading to glucose 6-phosphate build up, hexokinase II inhibition, improve within intracellular glucose content as a result, decrease in sugar uptake [45, 46]. Fatty acids build up intracellularly within myocytes primarily because long-chain fatty acyl-CoA, monoacylglcyerol, diacylglycerol, phosphatidic acid, triacylglycerol as well as ceramides. These types of fatty acid derivatives, high intramyocellular amounts of diacylglycerol, triacylglycerol, as well as ceramides tend to be related to insulin resistance [47, 48, 49, 50, 51]. It really is more developed in which the 1st step inside insulin's cell actions requires holding to certain necessary protein receptors situated on the plasma filters regarding targeted cells [17].

Hence, this indicates proper to begin with examining the particular cell

schedule regarding insulin level of resistance inside weight problems simply by emphasizing this kind of part of insulin's actions. Kahn et al have been first to examine the particular insulin receptor inside weight problems. The majority of overweight sufferers tend to be insulin proof, as well as numerous pet types of being overweight are also referred to by which insulin opposition is really a notable function. Reduced cellular insulin receptors happen to be noticed in a number of tissues through overweight animals as well as humans, and also the possible relationship in between reduced insulin receptors as well as insulin resistance is actually noticeable [18].

Almost all pet types of being overweight as well as insulin resistant analyzed and appear to create greater amounts of TNF/alpha/ mRNA as well as proteins. Neutralization research within overweight rodents having a soluble TNF receptor-IgG or even by way of gene transfer led to elevated insulin sensitivity, showing the participation associated with irregular TNF/alpha/ production within the insulin resistance of obesity.Current reviews additionally shown raised TNF/alpha/ mRNA as well as protein within human that are overweight. [19].

Recently, inhibition associated with insulin working as well as insulin receptor signalling by TNF/alpha/ had been shown within cultured human being adipocytes.These kind of results suggested that the working regarding to TNF/alpha/ inside individual having overweight problem is same as rodent models. A research utilizing a single dosage of the neutralizing antibody hasn't produced any changes in insulin sensitivity within individuals having type two diabetes. The result regarding TNF neutralization will not be noticed in almost all trial and error designs. Hence, TNF-/*alpha*/ might be a part contributor to insulin resistance [20]. Another procedure to the signaling disorders throughout over weight would be the greater manifestation along with task involving numerous health proteins tyrosine phosphatases (PTPs), which in turn dephosphorylate thereby stop signaling propagated by way of tyrosyl phosphorylation situations.

A number of files suggest that will at the least about three PTPs, which

include PTP1B, leukocyte antigen related phosphates (LAR), along with src-homology-phosphatase 2, are generally greater throughout manifestation and/or task throughout muscles along with adipose tissue involving fat individuals along with animals [21]. PTP1B and also LAR are already shown to dephosphorylate the particular insulin receptor and also IRS-1 inside vitro. This indicates the regulating part with regard to PTP1B not just within insulin motion, but additionally within power homeostasis. The actual insulin sensitivity exists within muscles as well as in liver although not within adipocytes [22]. Figure 2.3 The diagrammatic representation of Insulin resistance pathway obtained through KEGG database.

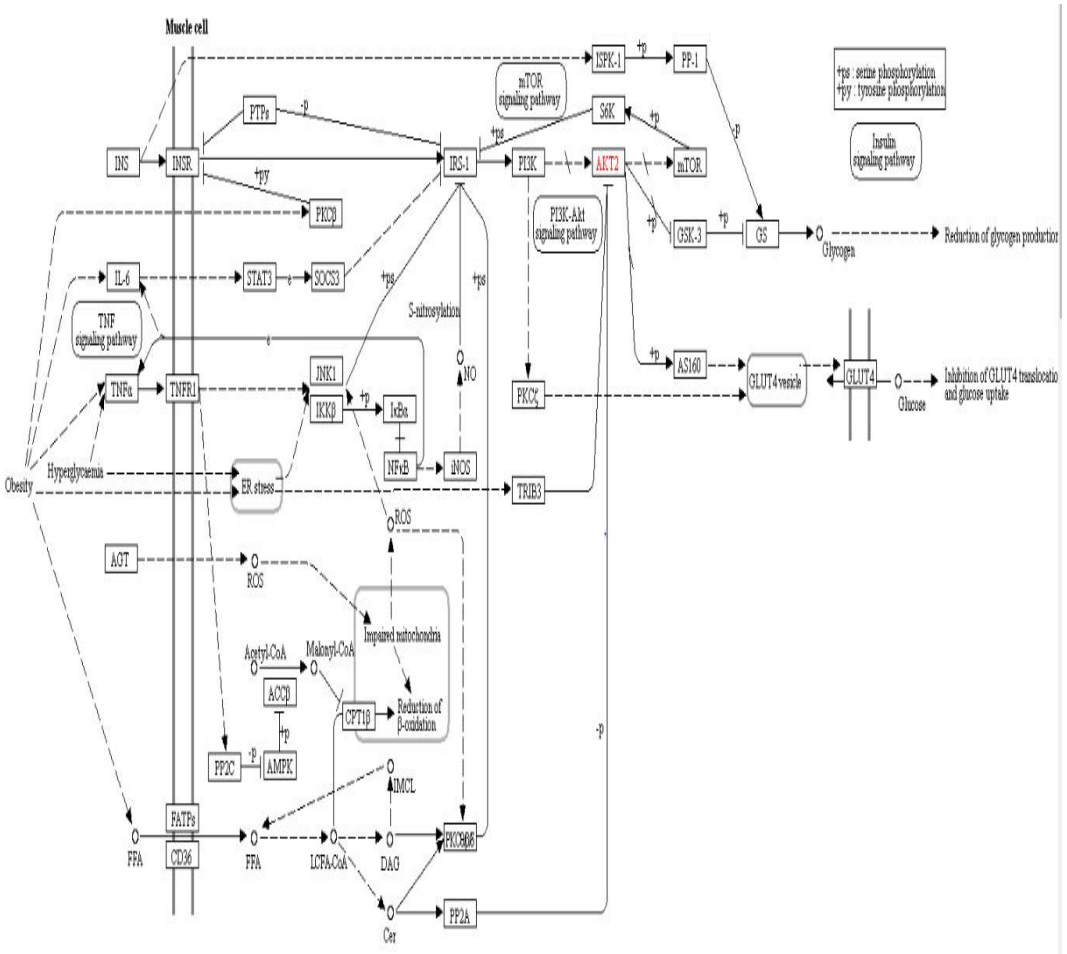


FIGURE 2.3: Insulin Resistance Pathway

2.7 Formal Modeling

Biological systems have different entities these entities are represented in the form of nodes in a pathway and connecting them with the help of interactions. These interactions are positive or negative that can provoke the other entity or inhibit and that makes a huge biological system. Systems are very complex and difficult to understand so scientists have given different approaches to understand them by using different strategies to analyze and understand complex pathways with the aid of reductionist approach. Researcher used reductionist approach to remove useless data that make the system much noisier and affect the analytical capabilities negatively. Some of the techniques which are of prime interest for researchers and have been used intensively that include Qualitative modeling as well as Quantitative modeling, unraveling the dynamics of the system [52].

2.7.1 Qualitative Modeling

Qualitative modeling strategy handles the actual rendering of the program within conditions qualitative qualities, that may also be used in order to subjective quantitative information too. The actual qualitative modeling particular function flow depends on graphical concept for that visual rendering from the program. Graphical concept is really a numerical field regarding the actual rendering associated with information within the shape associated with connections in between entities called as nodes, using the organizations symbolizing different items and also the connections represent the actual relationships in between individual's items [23].

From the later 1970s, Rene Thomas offered the qualitative formalism depending on Boolean reasoning relevant at first upon Gene regulating systems that carefully estimated their own particular ODE versions. Afterwards, it had been discovered that the actual Boolean design had been insufficient with regard to modeling different relationships which are present in GRNs from different gene levels, compelling Thomas to provide the actual kinetic reasoning formalism that

allows the actual modeling associated with discretely focus amounts over '1' to '0' and named it Biological regulatory network [24].

2.7.2 Quantitative Modeling

This kind of modeling can be helpful to identify the dynamical conduct from the pathway. This particular range from the constant condition, optimum concentration involved with a few modification, information related to time and so on. Numerous methods are utilized thoroughly with regard to this kind of evaluation from them Ordinary Differential Equation (ODE) modeling is actually popular [53]. Rate of modifications of every entity active in the pathway tend to be indicated as differential formula. These types of differential equations tend to be resolved concurrently to find the statistical answer. Different algorithms are utilized to resolve these types of equations. Different laws and regulations are utilized with regard to composing differential equations. Michaelis-Menten kinetics as well as law of mass action tends to be two well liked laws which convey the actual conduct associated with chemical substance responses within livings. These types of laws make use of quantity of constants that are experimentally decided requiring large number of wet lab resources [36].

TABLE 2.1: An overview of existing approaches of modeling.

Technique	Advantages	Disadvantages
Boolean Networks [63]	Boolean formalism represent realistic complex biological phenomena, like cellular state dynamics that work switch like behavior	The updates of the network states in this model are synchronous, where as biological networks are typically asynchronous.
Probabilistic Boolean Networks [62]	It is stochastic. They are able to cope with uncertainty	Even it is stochastic the state space is discrete.
Bayesian Network [56,57]	Effective in dealing with noise, incompleteness and stochastic aspects of gene regulation	Fail to consider temporal dynamic aspects that are very important in regulatory networks modeling.
Linear Model [61]	Linear models do not require much knowledge about regulatory network. It can be used to obtain qualitative insight about networks.	Failed to capture nonlinear dynamics (concentrations changing) of regulation.
Differential Equation based Model [60]	Simple homogeneous structures; This allows settings of parameter discovering software to be easily customized for these structures.	Involve a large number of parameters.
Single Molecule Level Model [59]	The most detailed, can capture stochasticity.	Computationally expensive
Hybrid Model [58]	Hybrid modeling helps in modeling both continuous aspects and discrete aspects.	Computationally expensive

2.8 Complexity of Modelling Biological Networks

Natural techniques frequently include complicated system associated with organizations that work together with one another to do regulating features. Because the amount of organizations inside a BRN improve, the actual computational needs to investigate this kind of internet functions turn out to be large. Actually with regard to little systems, creating versions with regard to many guidelines as well as analyzing every design towards some qualities is really a computationally difficult job. Model-checking methods need building as well as evaluation from the whole condition room of the design. Numerous methods happen to be created to deal with these types of restrictions. Using on-the-fly algorithms enables to deal with the issue associated with "state-space-explosion" through processing design condition room inside a series associated with actions.

Using "Binary-Decision-Diagrams or even BDDs"enables in order to encode design inside a storage effective method. The actual design dimension could be decreased by using incomplete purchase decrease algorithms. These types of design decrease as well as wise development methods assistance to deal with intricacy somewhat, nevertheless it is the situation which actually the actual decreased versions tend to be scanned with regard to high number associated with design guidelines. Because of the beginning associated with multicore processors, using High end Processing (HPC) offers surfaced like a guaranteeing strategy to deal with the actual intricacy associated with natural techniques.

Chapter 3

Material and Methods

3.1 Tools and Technologies Used

3.1.1 Kegg

For Retrieval of Pathway

3.1.2 Genotech

For Qualitative Modeling

3.1.3 Ginsim

For Qualitative Modeling

3.1.4 SMBionet

For Formal Model Checking

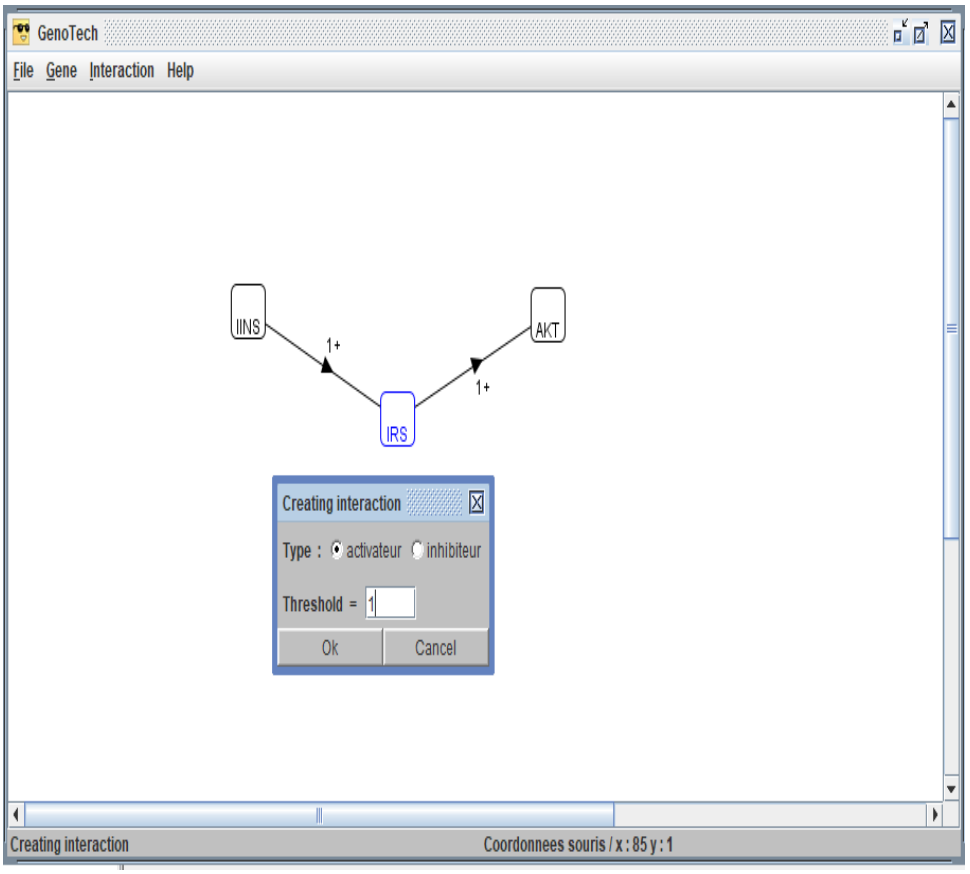


FIGURE 3.1: User interface of Genotech.

3.1.5 NuSMV Model Checker

For Model Checking

3.1.6 Cytoscape

For Network Analysis

3.1.7 HyTECH Model Checker

For Computation of Time Delays using Bio-LHA

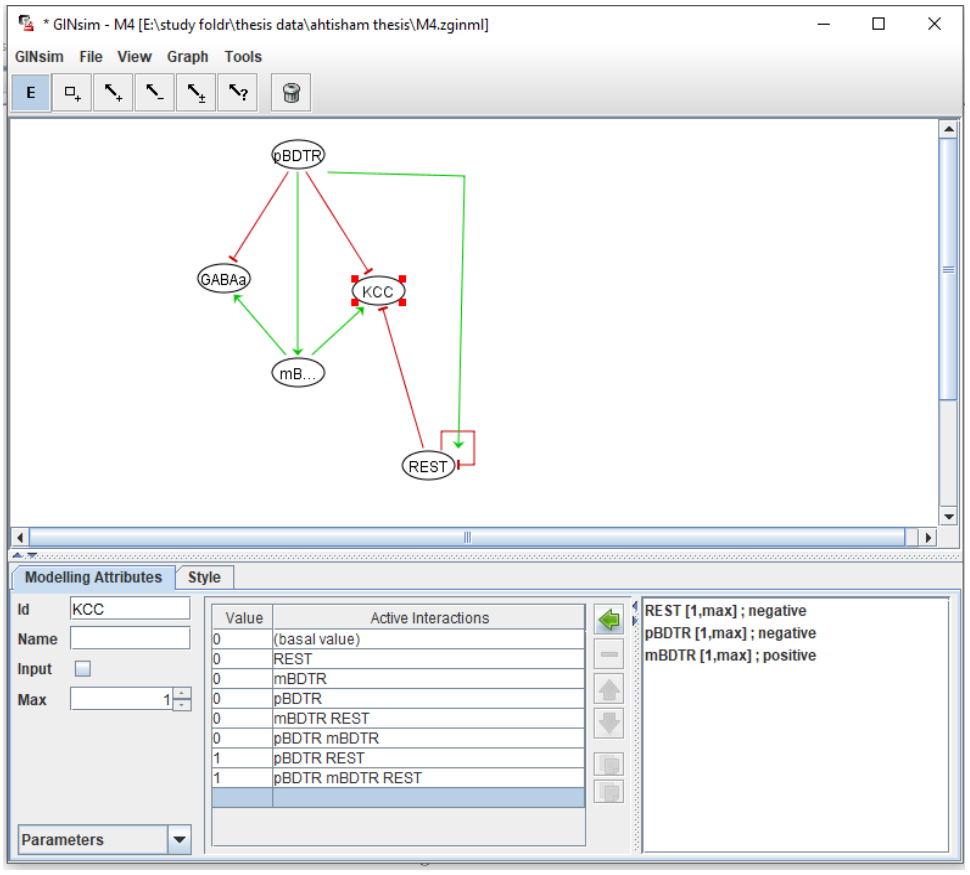


FIGURE 3.2: User interface of Ginsim.

3.1.8 SNOOPY

For Petri Net Modeling

The methodology of this study, begins with the qualitative modeling carried out through the use of the actual kinetic reasoning formalism associated with Rene Thomas', referred too within his book Biological Feedback [24]. The particular created qualitative product will be next changed into hybrid model while using the Bio-LHA platform produced and also discussed in [25]. Then that model will be converted in to Petri Nets [26]. Figure 3.3 will illustrate the whole methodology. The work starts with the pathway extraction and literature review symbolized with blue color with the help of different databases used for biological pathways e.g KEGG, Reactome etc, then the research initialized using the qualitative modeling, symbolized the area along with indigo color in which first step is construction of BRN then that BRN will be used to identify logical parameters with the help of

SMBionet and NuSMV tool, after parameters estimations we will use Genotech/-Ginsim tool to extract state graph and will use that state graph with the help of Cytoscape tool to extract important trajectory which leads to a deadlock state on the basis of betweenness centrality. After that we will move towards hybrid modeling symbolized along with yellow color and for that we use HyTech tool, as well as finished along with quantitative modeling symbolized along with green color and for that we will use Snoopy tool.

3.1.9 Methodology

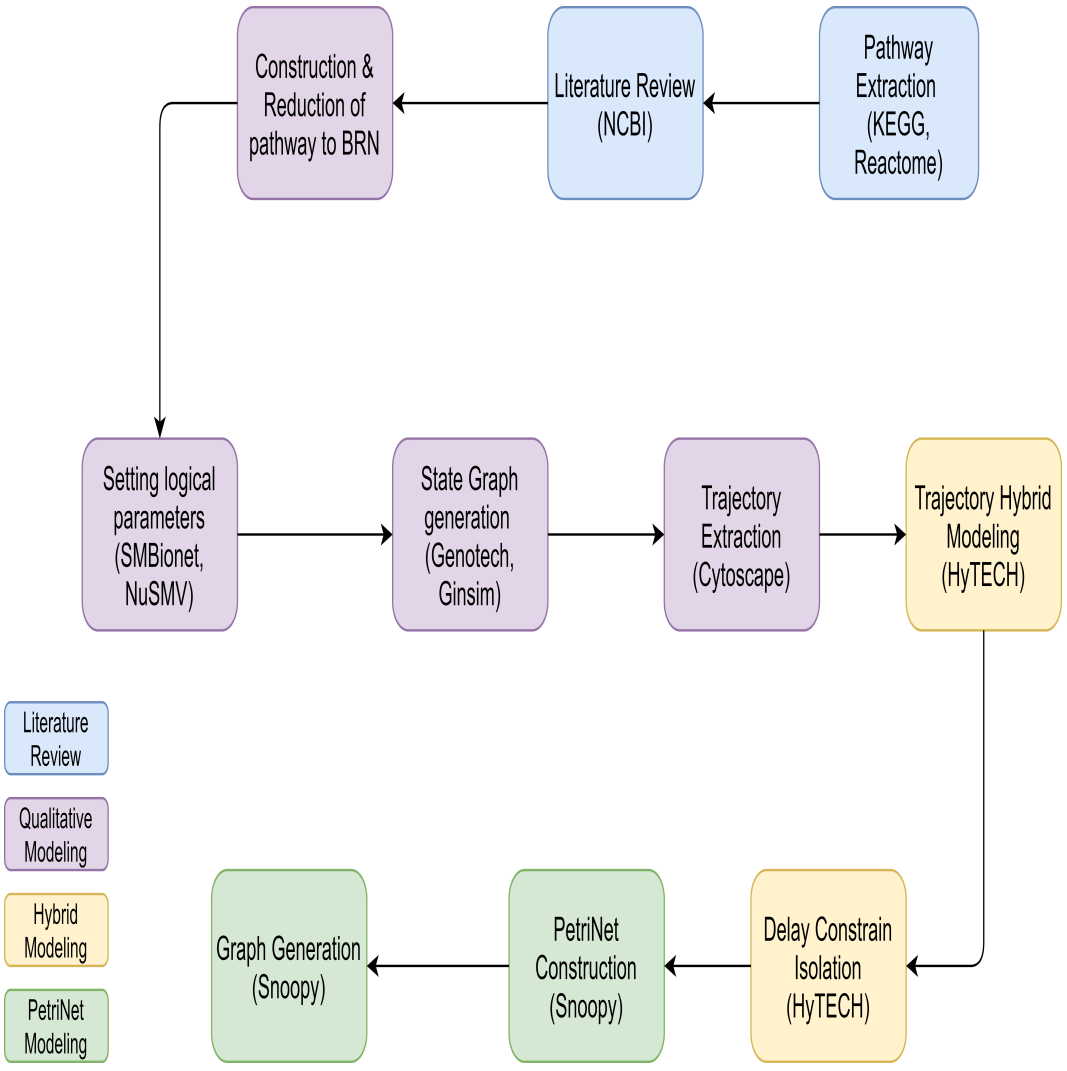


FIGURE 3.3: Methodology

3.2 Exemplary Scenario

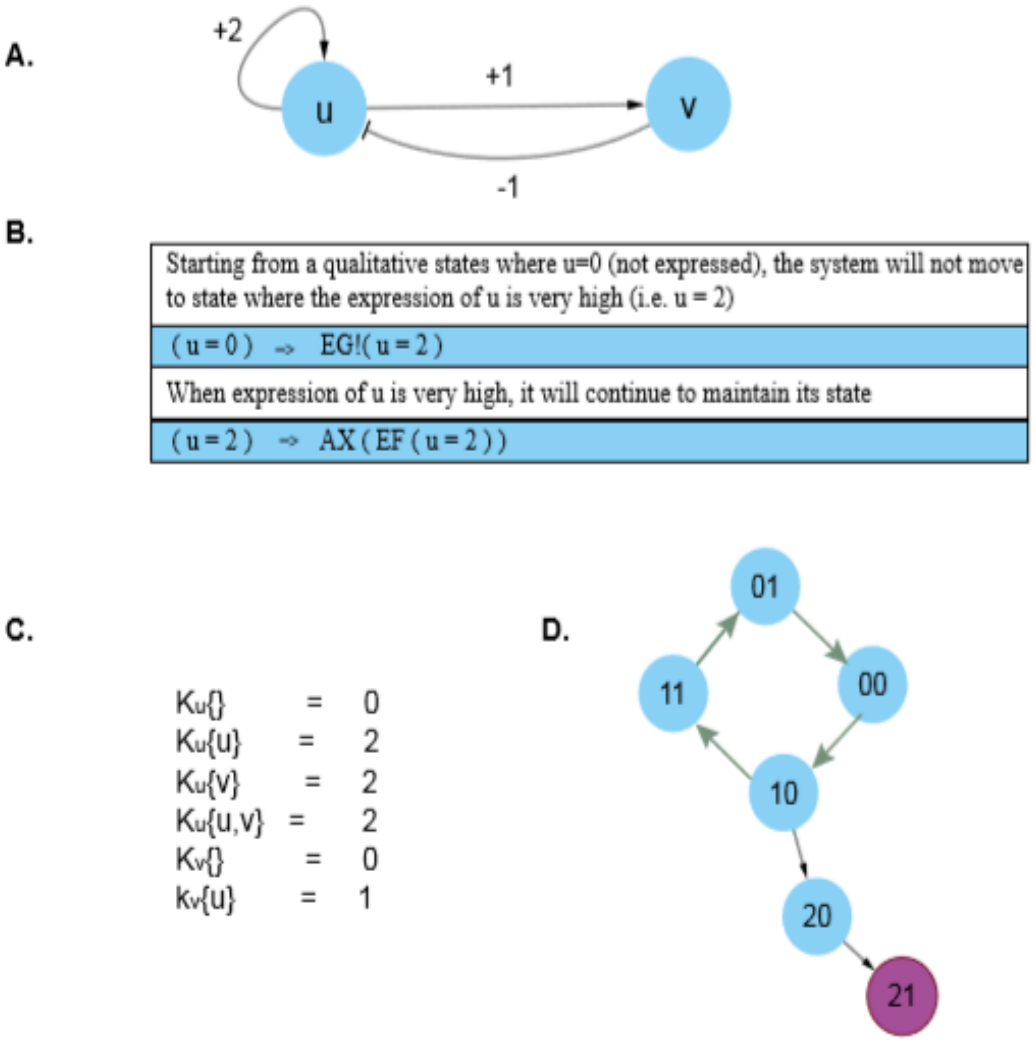


FIGURE 3.4: Qualitative modelling used upon BRN which regulates mucus manufacturing within Pseudomonas Aeruginosa.

To be able to clarify the actual operating associated with qualitative modeling, we use a good example of the BRN associated with pseudomonas aeruginosa- a pathogen generally present in atmosphere as well as accounts for mucus production within human being lung area. The actual BRN includes 2 organizations; the gene ALGU symbolized through X and inhibitor protein symbolized as Y. Both entities X as well as Y work in the following manner as given in Figure 3.4. Activations tend to be proven along with directed arrow tagged along with "+" indication. The actual inhibitions tend to be proven having a inhibitory arrow tagged along

with "-" indication. The gene X on service, favorably activate the Y. This means whenever phrase degree of gene X increases through its basal degree (0), as well as gets to at least 1, this triggers the actual functionality associated with Y. The actual conversation chart exhibits structural topology associated with 2 genes however it doesn't show the way the BRN may changes as time passes. For instance, in a specific period immediate, phrase amounts of gene X as well as Y could be from their own basal degree, as well as condition associated with BRN could be symbolized with a vector [0,0]. The actual character of the BRN tends to be symbolized through state graph that is produced through some logical parameters. These types of guidelines tend to be computed using a model-checker. To be able to calculate these types of guidelines, known observations usually are translated into a temporal reason data format. The actual model checker iterates via just about all feasible combinations as well as chooses parameters that fulfill temporary reasoning format then these kinds of parameters are next utilized to create state graph.

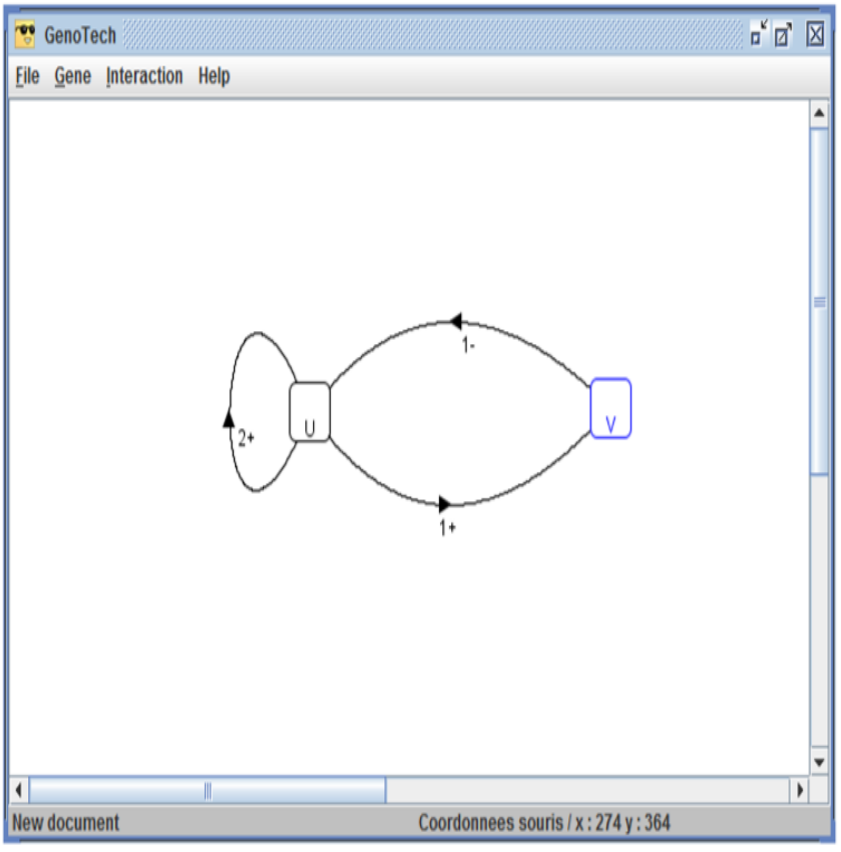


FIGURE 3.5: Design within GENOTECH Software.

Qualitative modeling can be executed by utilizing various software program deals for example GENOTECH shown in figure 3.5 The actual GENOTECH software program offers Graphical user Interface (GUI) with regard to development associated with qualitative model. The actual design is done by utilizing nodes as well as relationships about the canvas given in(A) as well as GinSIM figure 3.10 shows GinSim software user interface. GinSim software offers a user friendly interface with regard to design and exploring. The connection model is made canvas along with logical parameters. Figure 3.6 shows Activating as well as Inhibitory thresholds are specific to each connection. Logical parameters are given by double-clicking on each entity. Each resources supply simple to use options for model making, setting parameters related to logics and study of the state graph. Figure 3.7 shows image associated with state-graph within GENOTECH Software. The actual GENOTECH software program offers numerous choices to investigate the actual state-graph for example recognition associated with deadlock states, view of cycles as well as look at route of cycles. The image shows 1 deadlock condition (2, 1) and a cycle having 4 qualitative states (0, 0), (1, 0), (1, 1) as well as (0, 1)

3.3 Reducing Biological Network

The state space associated with qualitative model increase tremendously along with increase within quantity of entities. Naldi et al in [34] created the decrease way of lowering dimension associated with qualitative model whilst protecting their topological as well as dynamic features. Saadat pour et al offered the decrease way of Boolean systems which not just maintains the actual steady states but additionally maintains attractors [35].

The particular method introduced simply by Saadat et al. may be presented with the aid of a straightforward illustration. As an example, when a thing “A” stimulates the particular activity regarding one more thing “B” which usually more stimulates “C”, next through the use of the particular lowering method

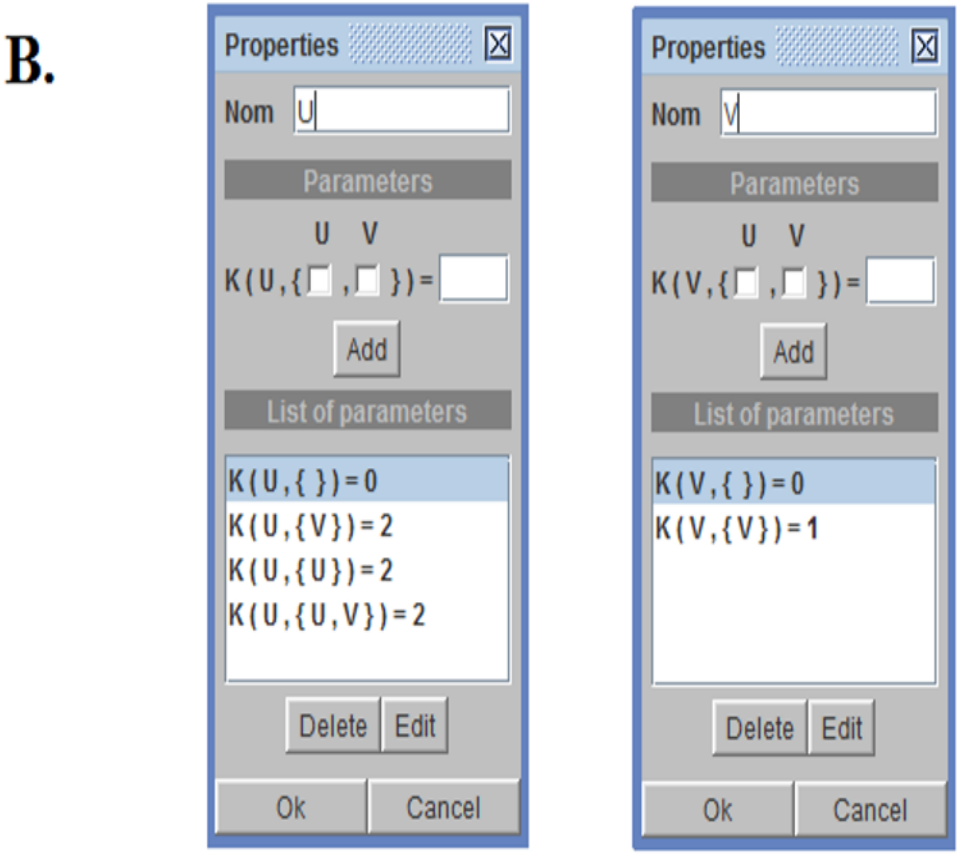


FIGURE 3.6: Activating as well as Inhibitory thresholds specific to each connection.

introduced simply by Saadat et al. the particular thing "B" may be taken out from the system. Saadat pour et al. utilized their particular lowering method on abscisic acid signaling pathway regarding thirteen entities and also lowered the particular community to be able to simply a few entities as demonstrated in Figure 3.8.

3.4 Formal Modeling

The actual dynamics associated with Thomas's technique tend to be produced through converting the traction graph to some condition state graph utilizing some reasonable logical parameters that are unfamiliar at the start. The actual evaluation associated with model parameters constitutes an essential part of qualitative modeling associated with systems. Bernot et al. [37] launched a solution to

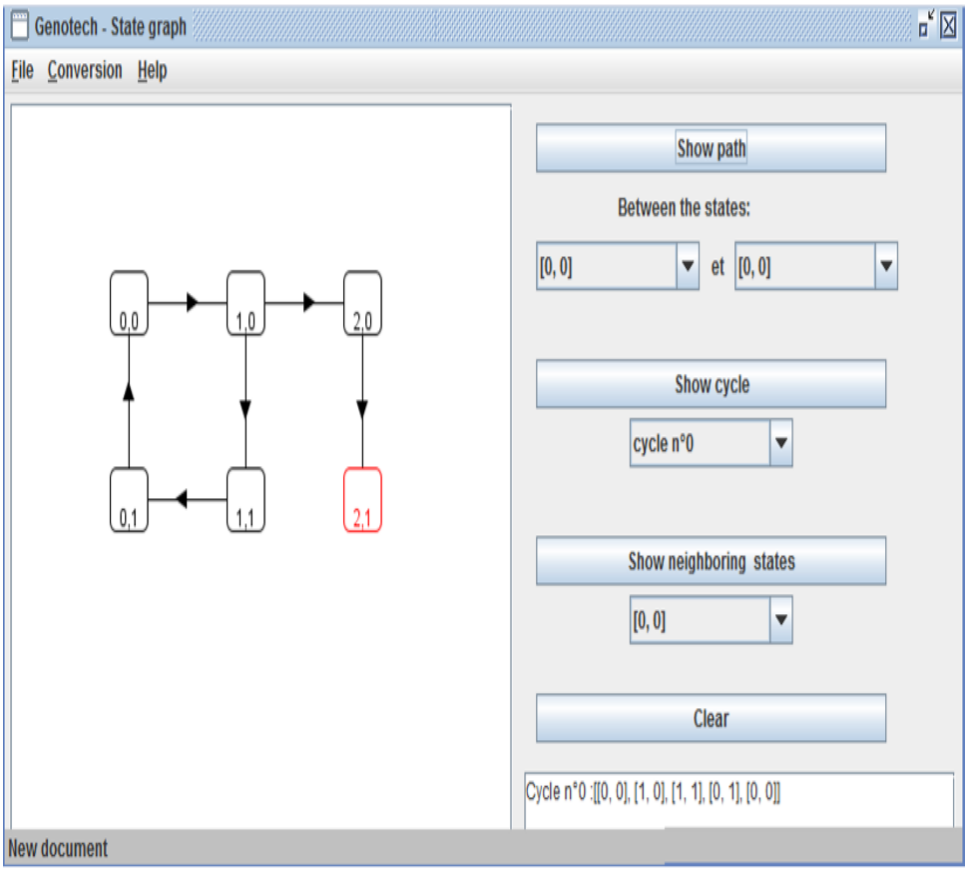


FIGURE 3.7: Visual image associated with state-graph within GENOTECH Software.

decipher these types of guidelines by using the formal verification strategy, known as model checking.

With this strategy, recognized experimental findings tend to be encoded inside a temporal reasoning framework, known as computational tree logic (CTL), after which while using model checker, various parameter combination tend to be examined in order to choose parameters that fulfill CTL observations. In CTL, experimental findings tend to be encoded in to formulations using a group of quantifiers that determine requirements in order to discover various states or even pathways via given state. A brief explanation of quantifiers and semantics is provided in [38].

Definition 1

Graph

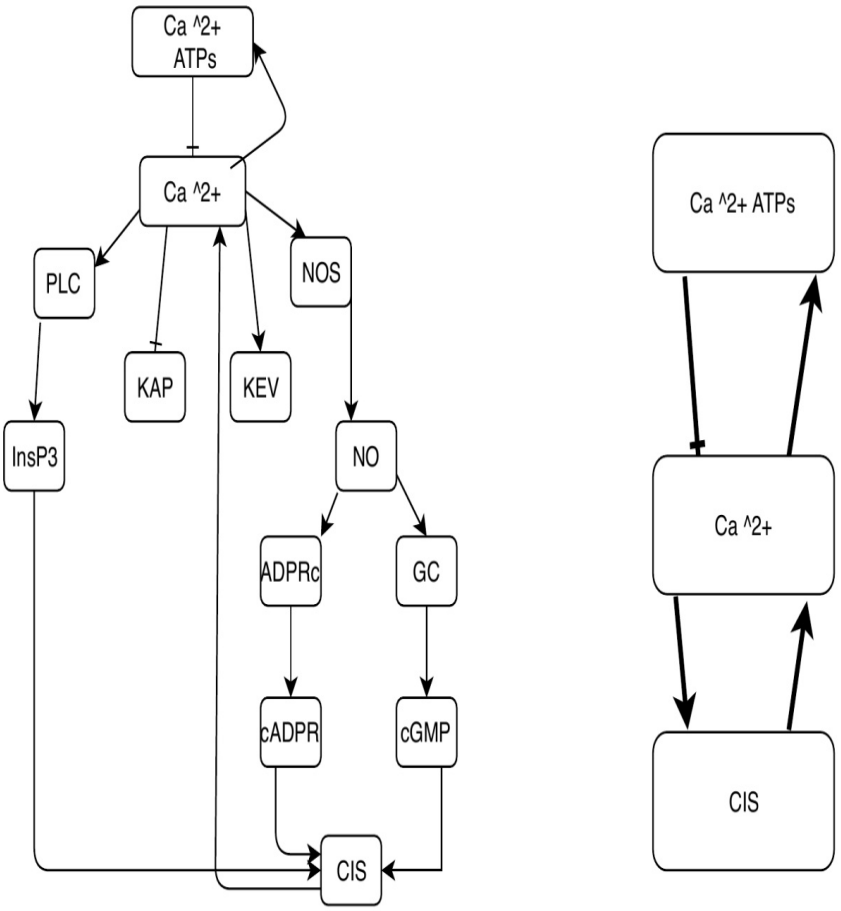


FIGURE 3.8: Abstraction of Qualitative Models adapted from Saadat pour et al [35].

Graph G is a tuple G = (V,E) where,

- V having element e, represents set of vertices
- E, having a typical element, is the set of edges representing the connections between the vertices.

.

· Definition 2

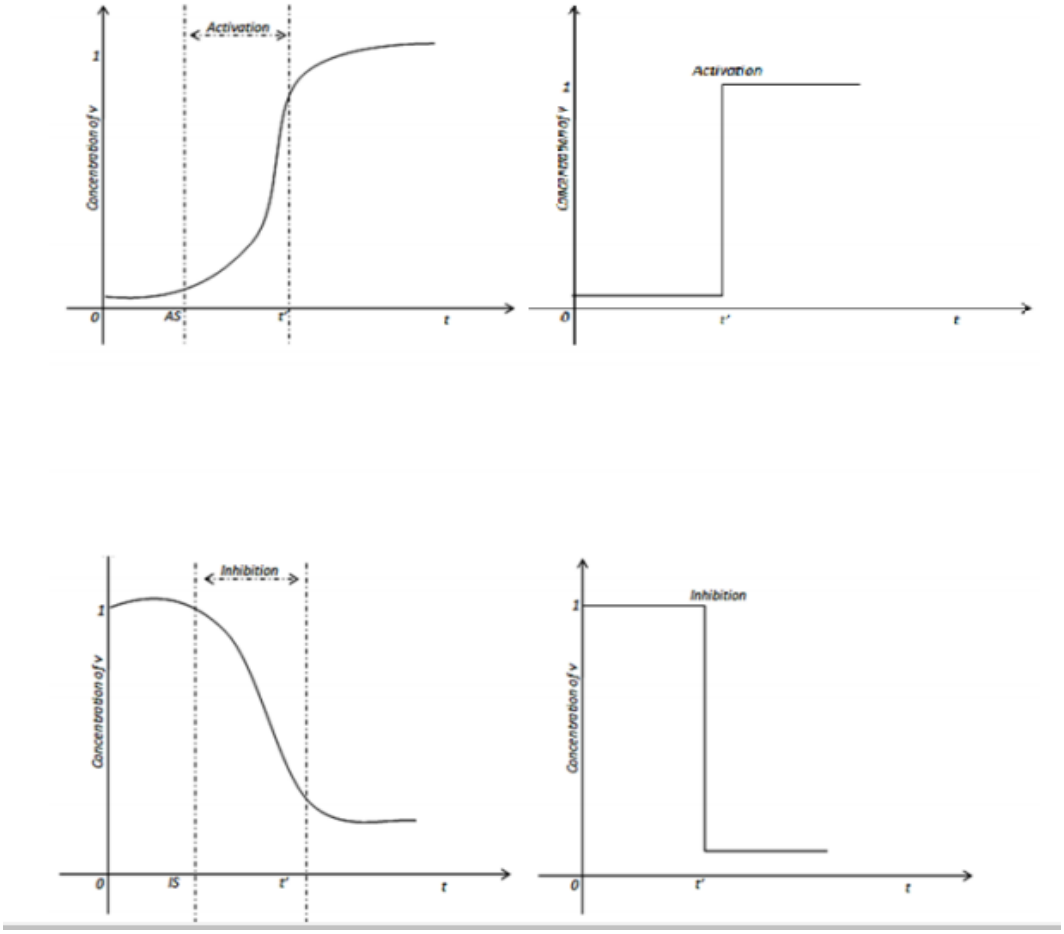


FIGURE 3.9: Discrete and continuous concentration of x. AS (activating) and IS (inhibition) signal in sigmoid curves.

Degree

A level of a vertex v is defined as the quantity of edges that a vertex holds with different vertices of the graph. For coordinated graphs, the degree property is part into two structures

- In-degree: they refer to number to incoming edges.
- Out-degree: they refer to number of outgoing edges.

.

Definition 3

BRN

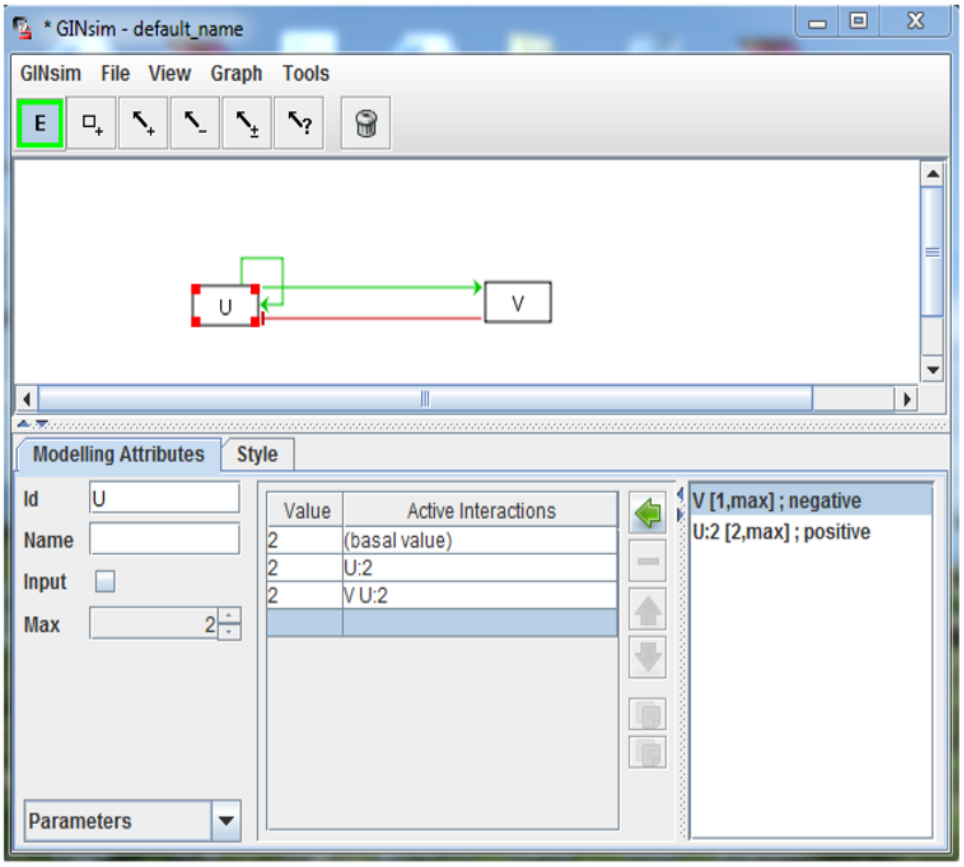


FIGURE 3.10: Qualitative design within GinSim Software.

A graph G = (V,E) is a biological regulatory network where, V represent entites and E represent connection between them. Each of the edge has two components, (τ) which represents the threshold value on which gene one starts activating the other, (Sigma) which represents the type of interaction either positive showing activation or negative showing inhibition.

- Where (τ) show the abstracted value of the concentrations.

Definition 4

Resource

A BRN $B = \{Dg, x\}$ is a directed graph $Dg = \{E, C\}$,a resource set $Rs_{(e_j)}$ has e_i which acts as resource of e_j when, there is a state $S_{(e_i} \in S$ and edges $e_i \Longrightarrow e_j$ associated with threshold T and label L . Mathematically it can be written as;

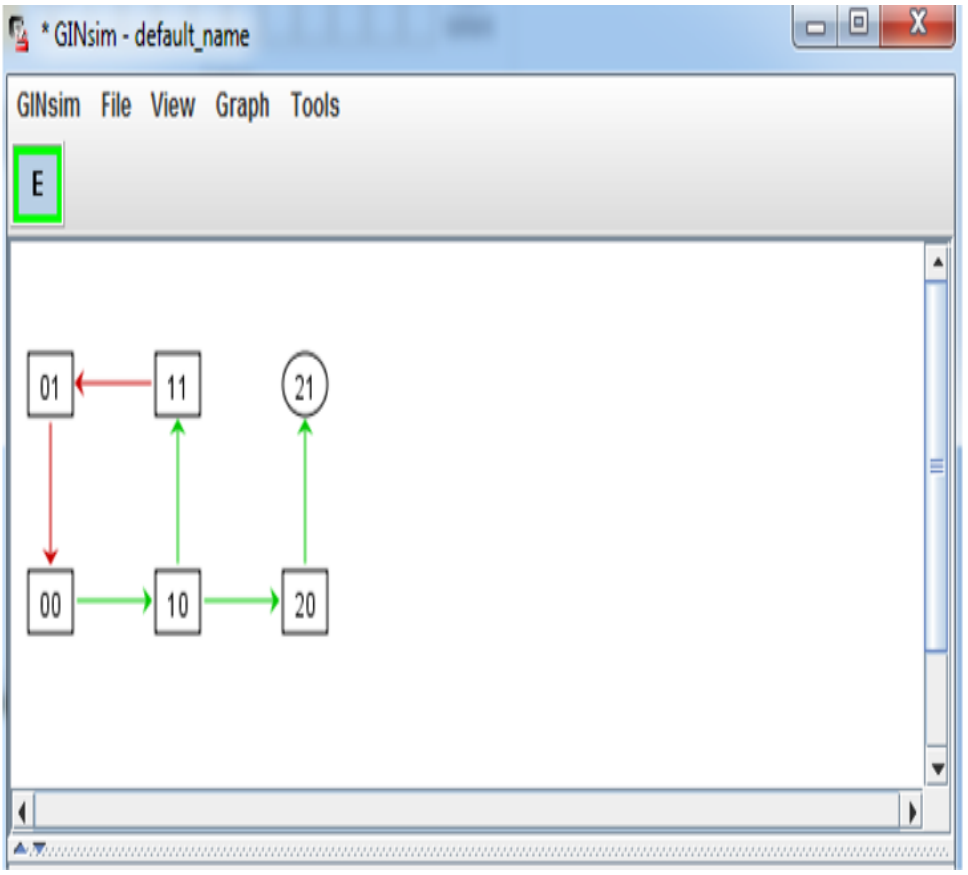


FIGURE 3.11: The GinSim software is used to produce state transition-graph.

$Rs_{e_j} = \{e_j \in Dg_{e_j}\rceil(S_{ei} \geqslant T_{ei}T_{ej} \wedge \mathfrak{G}_{e_i,ej} = "+") \vee (S_{ei} < T_{ei}T_{ej} \wedge \mathfrak{G}_{e_i,ej} = " - ")\vee)\}$

There is a mathematical trap for the resources said that asset is said to be available if activator is available or inhibitor is missing. Taking into the model situation where M can act as a resource of Z particles just for the situation when its esteem is under 1, which in real is 0. $Rs_{(e_i)}$ set contains activator and inhibitors on the basis of which variables e_i evolve. These variables are called as logical parameters i-e $Lp_{(e_i)}(Rs_{(e_i)})$ and defined under.

Definition 5

Logical Parameters

Logical parameters Lp for a BRN i-e $B = \{Dg, x\}$, can be defined as $Lp(\mathfrak{R}) = Lp_{ei}(Rs_{ei}) \in Z_{ei} \forall e_i \in E$ Evolution operator is used to map the discrete

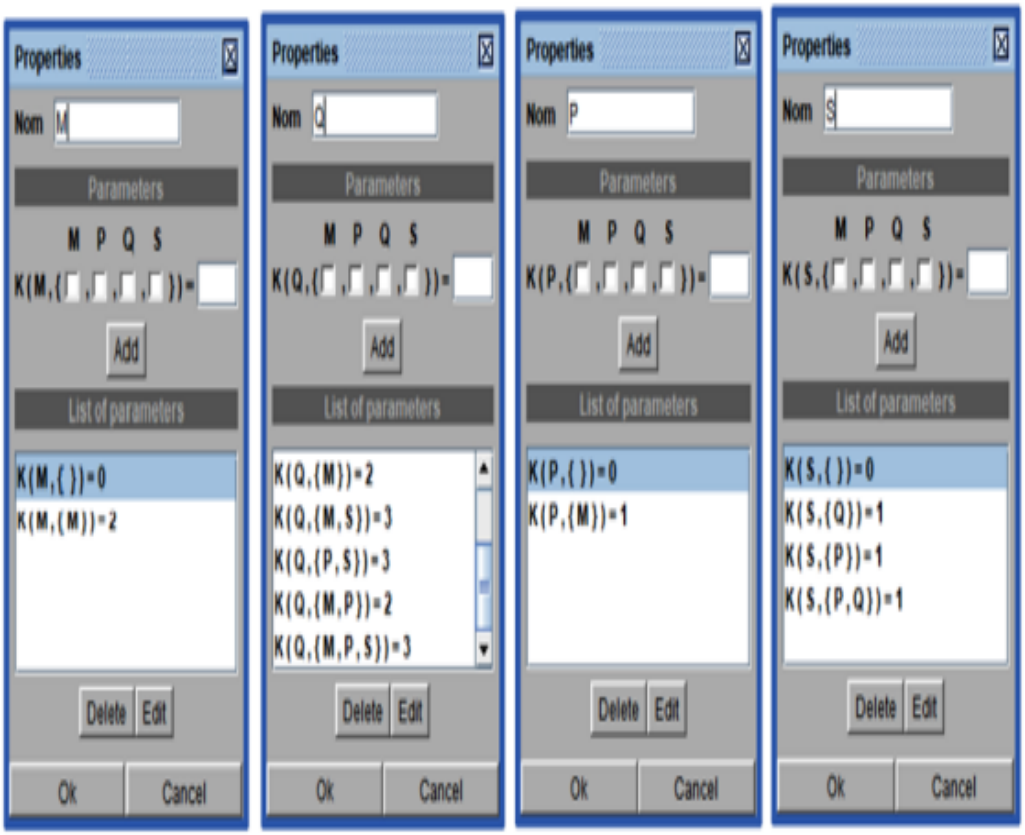


FIGURE 3.12: Assigning Parameter resources.

concentration $Lp_{(e_i)}$ which governs the concentration of e_i

$$S_{ei} \Rsh Lp_{ei}(Rs_{ei}) = \begin{cases} S_{ei} \Rsh Lp_{ei}(Rs_{ei}) = S_{ei} + 1 if S_{ei} < Lp_{ei}Rs_{vi} \\ S_{ei} \Rsh Lp_{ei}(Rs_{ei}) = S_{ei} - 1 if S_{ei} > Lp_{ei}Rs_{ei} \\ S_{ei} \qquad\qquad\qquad\qquad if S_{ei} = Lp_{ei}Rs_{ei} \end{cases}$$

Three possibilities have been shown in the equation given above i) When $S_{ei} < Lp_{ei}Rs_{ei}$, increment will occur in $S_{(e_i)}$. ii) When $S_{ei} > Lp_{ei}Rs_{ei}$, decrement will occur in $S_{(e_i)}$ and iii) when $S_{ei} = Lp_{ei}Rs_{ei}$, which means the value of $S_{(e_i)}$ remains same and doesn't evolve.

Definition 6

Trajectory

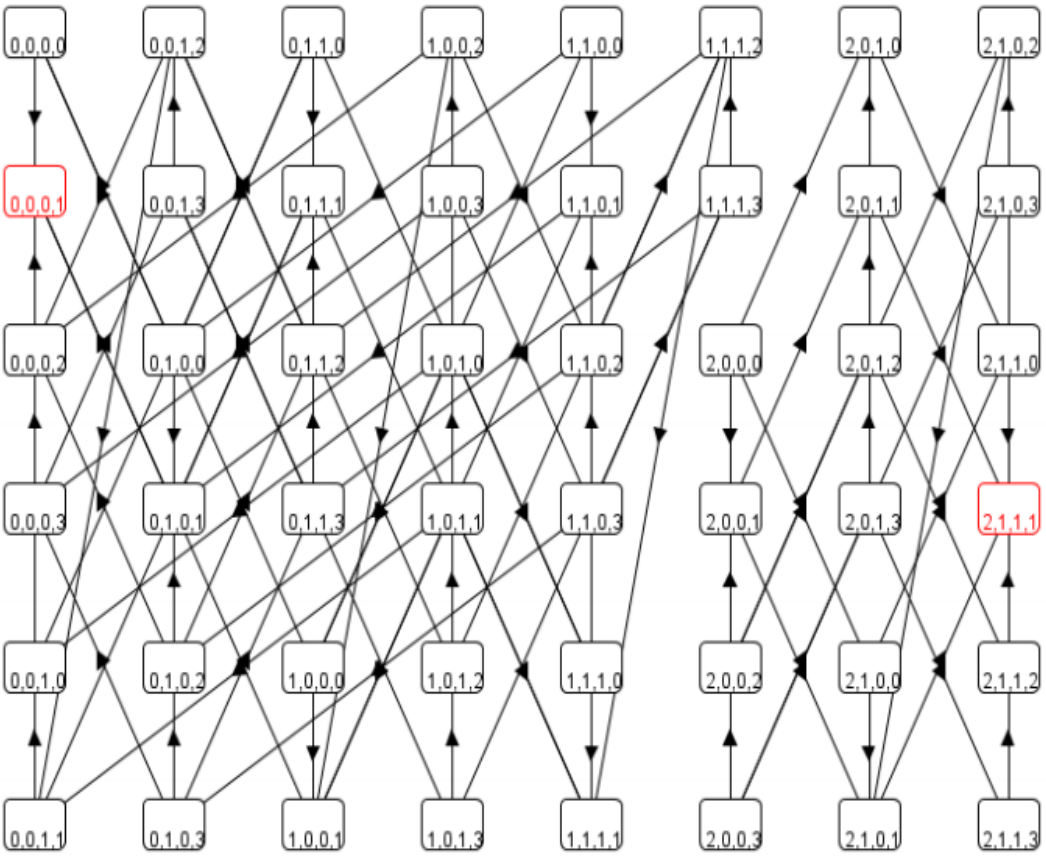


FIGURE 3.13: State graph of the example scenario computed in GenoTECH.

Trajectory represent the successive transition over a graph whereas cyclic trajectory always lead towards the initial state.

Definition 7

Radius

Radius is the shortest distance from set of shortest paths.

Definition 8

Diameter

Diameter, in contrast to radius, is the largest distance from set of shortest paths among states of a graph.

Definition 9

Efficiency

It represents the inverse of the diameter, where 0 represents the maximum diameter and 1 represents minimum diameter.

Definition 10

Closeness

It represents how far or near an entity lies to the other entity in a graph.

Definition 11

Betweenness

Betweenness represents the flow of information that takes place through an entity from the set of shortest paths from one vertex to other vertex in a graph.

3.5 SMBioNet

SMBioNet is a tool with regard to parameter inference associated with qualitative modeling of systems. It's based upon qualitative formalism associated with Rene Thomas as well as utilizes NuSMV [37] for model checking. SMBioNet will take a couple of inputs. The particular qualitative discussion graph and also pair of behaviour attributes (observations), portrayed as CTL, figure 3.16 shows the CTL. Experimanetal findings tend to be encoded into formulations using a group of quantifiers that determine requirements in order to discover various states or even pathways via confirmed condition. CTL quantifiers tend to be split in to path quantifiers (A, E) as well as state quantifiers (X, F, G) and also iterates by means of a selection a model through a range of given logical parameters which satisfy provided CTL[38].

3.6 Network Analysis

Graph Concept performs an essential part within modeling as well as evaluation associated with systems biology [26; 27; 28]. The actual graph-theoretic methods are utilized to investigate topological as well as structural parameters associated with natural systems to find out crucial qualities that offer significant experience to the performance associated with system. Identification of essential nodes inside a big regulating system is crucial to understand association with in systems.

The actual most favored calculate in order to calculate the actual position associated with nodes within graph-theoretic design, in line with the idea of centrality [28; 29], primarily result from Social networking Evaluation [30]. Centrality Evaluation has additionally already been used to study essential qualities associated with complicated natural regulating systems [31].

3.7 Hybrid Modeling with Delays

Discrete modeling gives use of complete information directly into qualitative characteristics regarding system. Nonetheless, increase or perhaps decrease in protein expression explained by way of a stage operate just isn't coherent together with genuine adjustments of protein expression occurring inside the cell. The actual focus degree of the proteins, for example, will not really jump from one discrete value to other.

To be able to catch the actual changes associated with proteins expression was introduced by Ahmad et al. this new method depending on piece wise linear equation. On this platform they modeled states of system as discrete location. In addition, particular factors, referred to as clocks, are employed to be able to designate limitations regarding move from individually distinct place to a new. Right here, they offer a short explanation associated with hybrid modeling construction, modified through [32, 33]. Time delays in between 2 consecutive qualitative

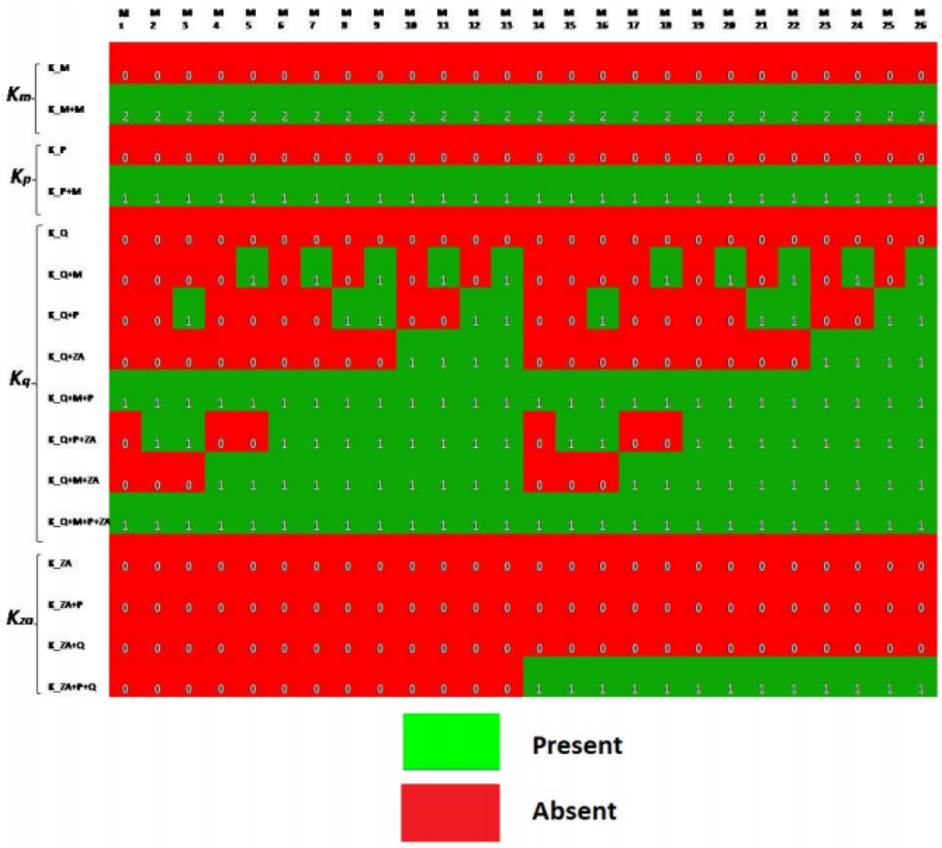


FIGURE 3.14: Representation of resources. Green color represents the presence and red color shows absence of resource.

expression amounts tend to be calculated through time clock parameters. The actual delay explains time lapse in between 2 qualitative states. For every protein within the BRN, the time clock adjustable is done as well as arranged in order to qualitative expression is "0". After that it reaches to d+ or even d-. The actual manufacturing delay related to a protein within BRN is actually distributed by d+. The actual manufacturing delay for every entity within the hybrid design explains time lapse to improve the actual expression degree of entity through "1". In the event of the Boolean system, this means time necessary to alter expression level in entity from 0 to 1. Likewise, the actual destruction delay related to a protein within the BRN is actually assigned by d-. For every entity within the hybrid design, the actual destruction delay explains the actual hybrid modeling along with delays period lapse within climbing down through greater qualitative expression to lower qualitative expression. For any Boolean system, this explains time necessary for changeover through qualitative phrase 1 to 0 [32].

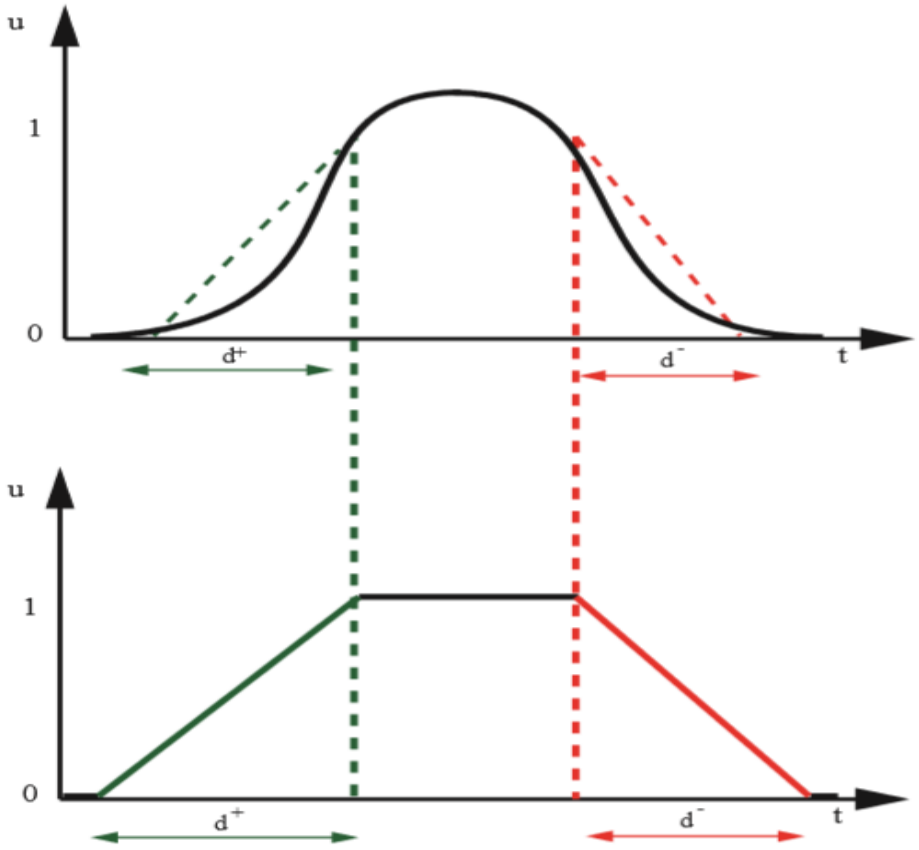


FIGURE 3.15: Activation and degradation delays of hybrid modeling presented as d"-" and d"+".

3.8 Petri Net Modeling

Using qualitative as well as hybrid modeling methods offers essential experience for example cycles, steady states as well as connection in between synthesis rate associated with entities. Nevertheless, these types of character stay under the radar because of the discrete character associated with qualitative modeling. Furthermore, biological systems tend to be random within character and may end up being symbolized much more precisely by utilizing stochastic PetriNet. The PetriNet can be viewed a good executable numerical graph that may be used to research character of the system composed of different entities communicating with one another. Right here, we offer Petri Net modeling with concentrate on Stochastic Petri Net. The detailed explanation of stochastic PetriNet is given in [39].Example of petrinet modeling is given in figure 3.17 including development of drinking water via oxygen as well as hydrogen. circles signify locations that are

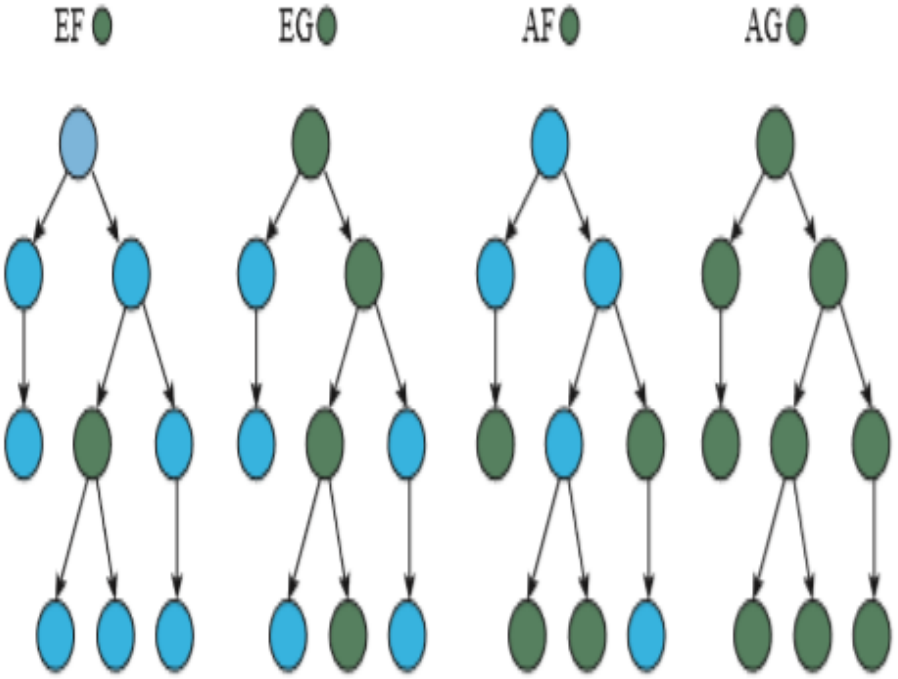


FIGURE 3.16: Computational tree logic.

accustomed to design reactants as well as items active in the chemical substance response. Figure 3.18 shows Petri net type of Kinetic response including development of drinking water through oxygen as well as hydrogen. circles signify locations that are accustomed to design reactants as well as items active in the chemical substance response. The actual tokens of the arcs shows stoichiometry from the chemical substance response leading to development of 4 water substances.

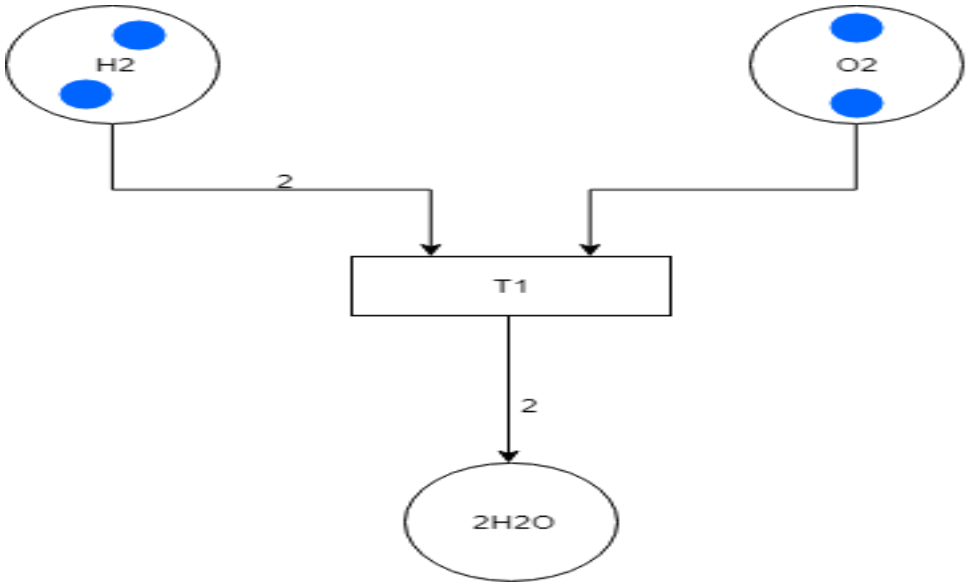


FIGURE 3.17: Example of petrinet modeling.

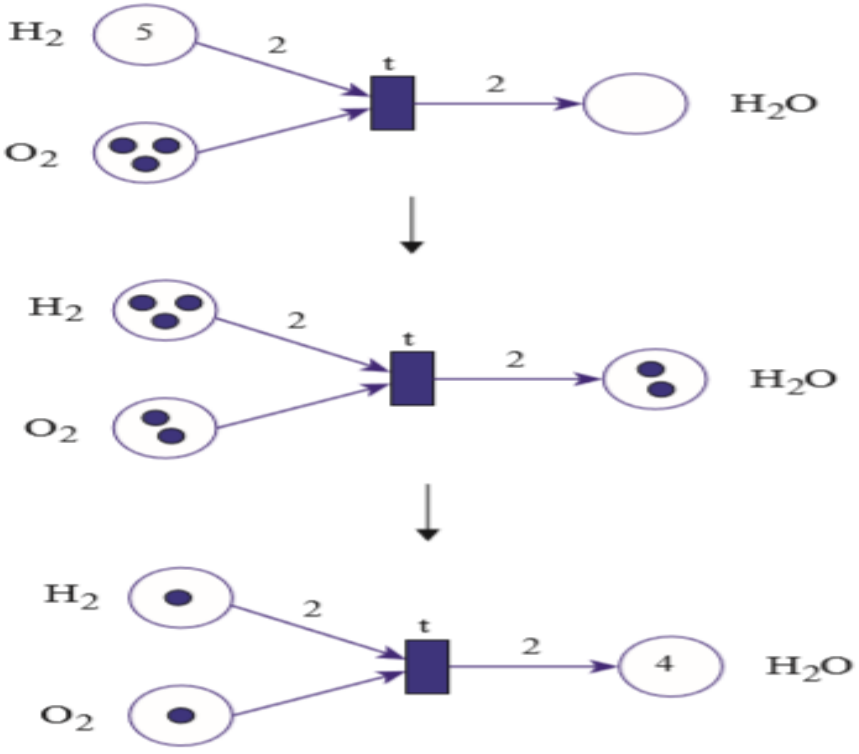


FIGURE 3.18: Petri net type of Kinetic response.

Chapter 4

Results and Discussions

This particular section uses the actual strategy described in the earlier section and applies it on Insulin Resistance Pathway explained in Chapter 2

4.1 Qualitative Modeling

From an intensive examine regarding present materials we abstracted a qualitative biological regulatory network. BRN was reduced as did in the previous studies by applying general rules e-g if entity “A” activates “B” which activates “C” then we can imply actually “A” is the entity which activates “C”. In addition to that those entities which had no source were also ignored in the analysis. Resulting reduced biological regulatory network includes 8 biological entities and 13 interactions in which 10 are negative and 3 are positive regulations. In a biological system feedback loops are of two kinds i-e positive feedback loops and negative feedback loops, researchers mostly focus on negative feedback loops as these are the root cause of oscillatory behavior in a graph and homeostasis in the biological system. In our proposed model we have only 1 negative feedback via IRS and akt. The analysis of these loops give proper insight to understand the dynamics of the system which are rendered with the help of formal modeling which provide the logical parameters to mine information from the state transition graph.

4.2 Biological Regulatory Network

BRN associated with Insulin Resistance pathway represent inhibitory action associated with INS, IRS as well as AKT. Natural feedback tend to be associated with perfect significance within the framework associated with paths or even within biological systems, because they take part in the oscillatory conduct of system or even they force the system in the direction of homeostasis.

Two types of Feedback loops are there, positive one having equal number of negative regulations where in the case of negative feedback loops there are an unequal number of negative regulations. Reduced biological regulating system includes 8 regulating entities as well as 13 various regulations covering 1 loop, 10 inhibitory regulations as well as 3 activating regulations. BRN offers 1 negative feedback cycle e-g AKT inhibits IRS which activates AKT. Reduced BRN was constructed on the basis of some rules mentioned earlier, which has total 8 entities while preserving the important regulatory oscillators or negative feedback loops shown in Fig 4.5.

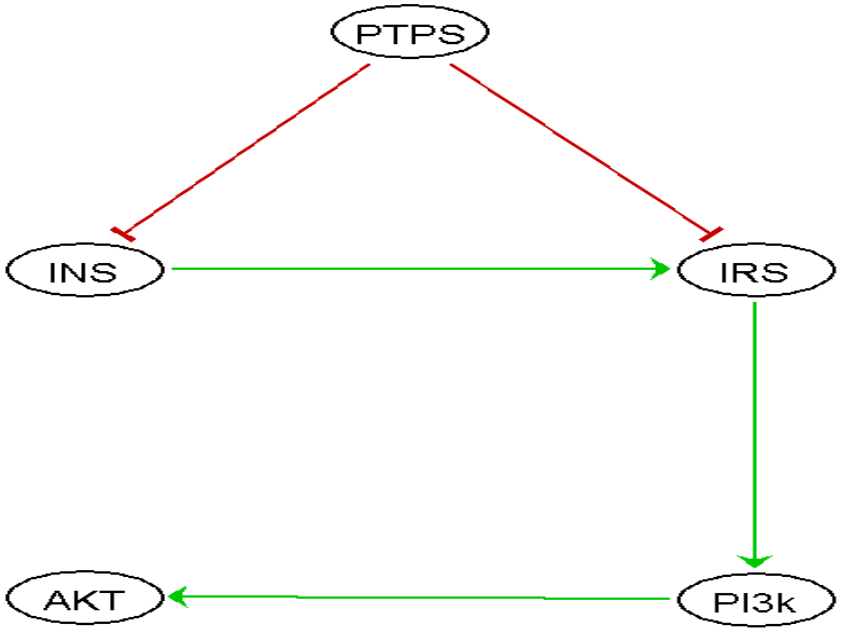


FIGURE 4.1: A reduced overall view of PTPs in Insulin Resistance Pathway.

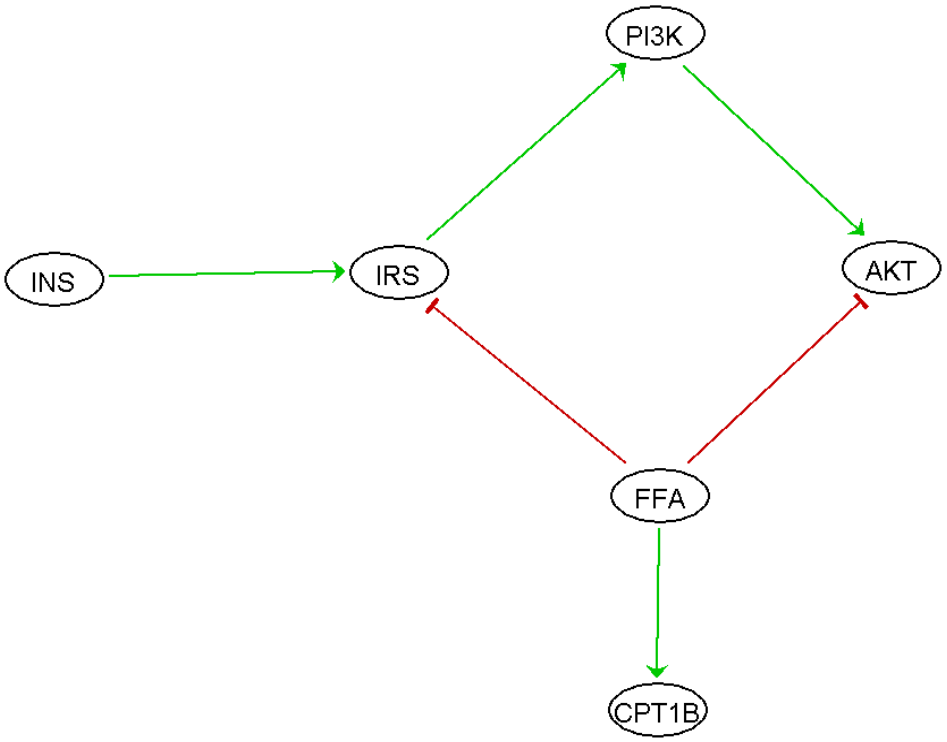


FIGURE 4.2: A reduced overall view of FFA in Insulin Resistance Pathway.

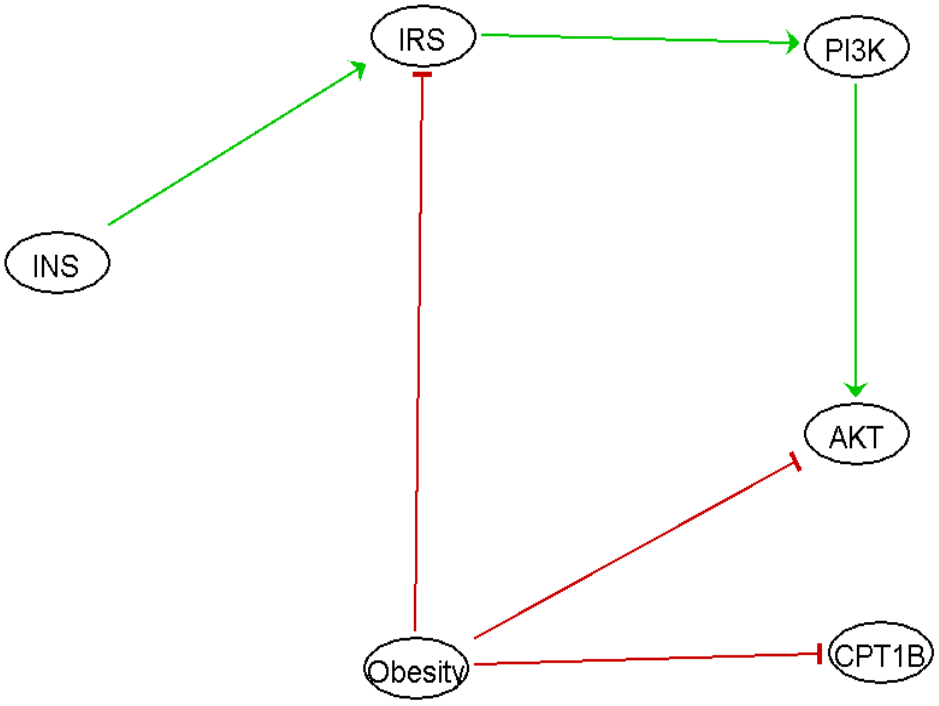


FIGURE 4.3: A reduced overall view of Obesity in Insulin Resistance Pathway.

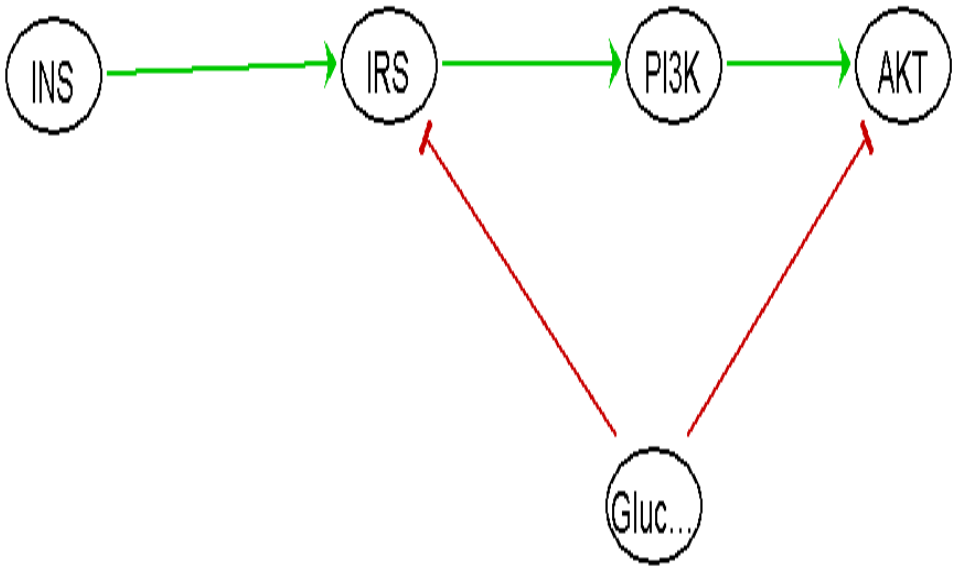


FIGURE 4.4: A reduced overall view of Glucose in Insulin Resistance Pathway.

4.3 Identification of Stable States

Steady state, quite simply fixpoints or even constant states would be the states which force the system in the direction where absolutely no alterations happens. Within logical modeling from the biological systems it's considered as loops tend to be straight related to constant state from the system in the direction of biostability or even multistationarity (64, 65). Regarding biological regulatory networks static evaluation is done to identify these kinds of states. In the case of Insulin Resistance Pathway, by the use of PINT process hitting framework, we were able to identify only one stable state. Table 4.1 represents the stable state of Insulin Resistance Pathway computed through the framework of process hitting which shows down regulation of INS, IRS and AKT present in Insulin Resistance Pathway.

The actual dependability from the steady state calculated depends upon the confirmation with the literature whether the steady state offers a significance or even not really important. Steady states associated with Insulin Resistance

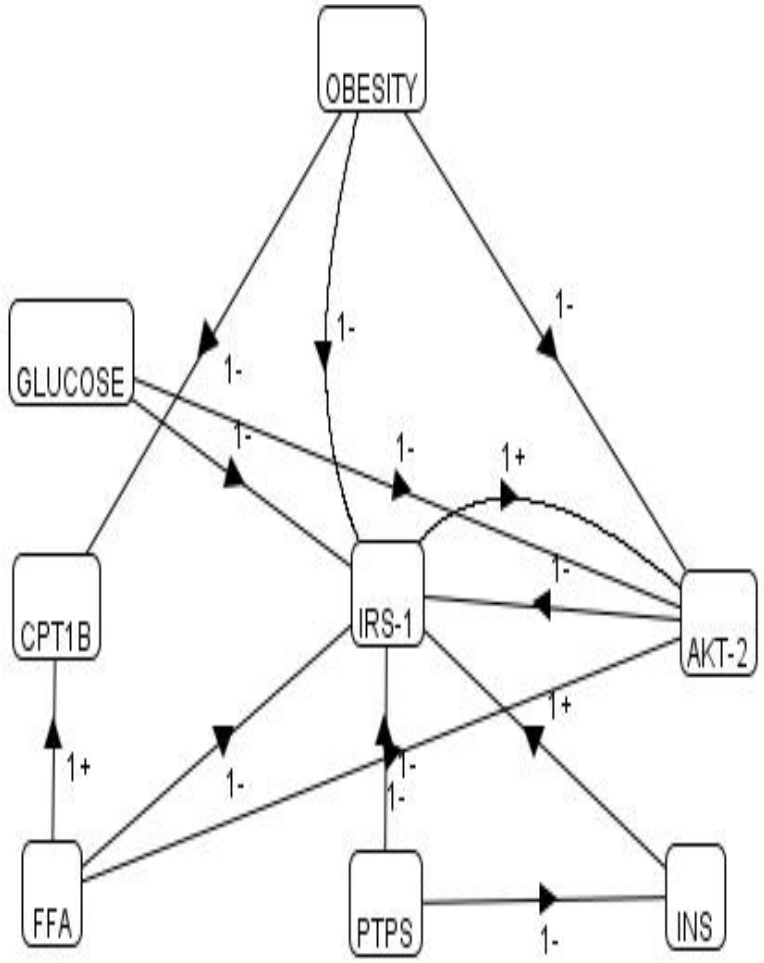


FIGURE 4.5: (BRN) A reduced overall view of Insulin Resistance Pathway.

Pathway' supports the literature that show inner rules associated with resistance associated with Insulin Resistance Pathway.

4.4 Computation of Logical Parameters

Logical parameters would be the situations which totally impact the actual conduct from the system. They were calculated through the usage of SMBioNet tool

that functions as well as discovers the actual model which essentially fulfill the inserted CTL formula which once deciphered show the biological observations of the system, as given in Table 4.2. CTL formula is actually encoded within SMBioNet in case that BRN' that have its power to recognize as well as get needed models.

TABLE 4.1: Stable state computed through framework of process hitting.

CPT1B	FFA	GLUCOSE	INS	IRS1	OBESITY	PTPS	AKT2
0	1	1	0	0	1	1	0

CTL encodes the steady state produced via process hitting framework which through initial system (1,0,0,0,0,0,0,0). Biological system standards such as it's variable, regulations as well as CTL formulations SMBioNet discovers the exact model combined with the logical parameters which take part in enforcement of the system in the direction of stable state as shown in Table 4.3 that are involved in enforcement of the system towards that stable state. First column represent the serial number, second column represent the parameter, third column represent the Resource sets, fourth represent Range and fifth represent selected logical parameters. After these steps we get 38 checked models in which 19 were selected as presented in figure 4.6 as heat map of Logical parameters in which the model shows the trajectories. The expression of parameters is represented with green rectangles whereas the down-regulated values are shown as orange rectangles on the basis of logical parameters and CTL formula. These types of logical parameters help with the generation of state graph leading towards the steady state from a large number of various trajectories from initial condition. After examine the Heat map reveals changing with in elements in the resource sets which cause either activation or degradation of a specific element. KAKT, KIRS and KINS are the important elements of the system that are down regulated by the presence of OBESITY, FFA, Glucose and PTPS. According to Rene Thomas KINS first recourse set is empty which means the presence of the inhibitor, in that event KINS moves toward 0 in the system.

TABLE 4.2: CTL formula encoded in SMBioNet.

CTL Formula	((INS=1&PTPS=0&IRS=0&AKT=0&FATS=0&GLUCOSE=0& CPTB=0&FFA=0) -¿ EF(AG(INS=0&PTPS=1&IRS=0&AKT=0& FATS=1&GLUCOSE=1&CPTB=0&FFA=1)))
Explanation	CTL formula checks the models which specify the stable state identified through process hitting.

4.5 State Transition Graph

Their state transition graph includes 128 nodes as well as 448 shown in figure 4.7 and it is made utilizing Cytoscape software. Their state graph is actually produced through selected logical parameters utilizing GENOTECH software as well as states are sorted based on betweeness centrality.

Within the state graph, their state from the system in a specific period is actually symbolized with a vector that contains expression level of all entities in the following order [INS, IRS, AKT, PTPS, OBESITY, FFA, GLUCOSE, CPTB]. The normal qualitative state in the state graph is characterized by low expression levels of IRS, AKT, PTPS, OBESITY, FFA, GLUCOSE and CPTB. Conversely, the diseased qualitative state is characterized by high expression levels of PTPS, OBESITY, FFA and GLUCOSE. The deadlock state (0,0,0,1,1,1,1,0) is a diseased state that shows low expression level of INS, IRS and AKT.

Because the total state graph generated from qualitative modeling is actually too much complicated to analyze every trajectory manually so we utilized the idea of average betweenness centrality to recognize important trajectories. All of the pathways through qualitative condition (1, 0, 0, 0, 0, 0, 0, 0) towards the deadlock condition (0, 0, 0, 1, 1, 1, 1, 0) had been examined, the path given in figure 4.8 had been chosen based on average betweenness centrality. Sub graph extracted from the state transition graph shows Insulin Resistance. Every node within the graph signifies a distinctive condition from the system seen as a qualitative expression of protein in the following order: (INS, IRS, AKT, PTPS, OBESITY, FFA, GLUCOSE, CPTB). Activation of a particular entity is represented

TABLE 4.3: Logical parameters.

S.No	Parameter	Resource Sets	Range	Selected	S.No	Parameter	Resource Sets	Range	Selected
1	KINS	{}	0	0	46	K-IRS	{AKT,FFA,INS,PTPS}	0	0
2	KINS	(PTPS }	1	1	47	K-IRS	{AKT,FATS,FFA,INS}	0	0
3	K_PTPS	{}	1	1	48	K-IRS	{FATS,FFA,GLUCOSE,INS}	0	0
4	K-IRS	{}	0	0	49	K-IRS	{FFA,GLUCOSE,INS,PTPS}	0	0
5	K-IRS	{AKT}	0	0	50	K-IRS	{FATS,FFA,GLUCOSE,PTPS}	0	0
6	K-IRS	{FATS}	0	0	51	K-IRS	{AKT,FATS,INS,PTPS}	0	0
7	K-IRS	{FFA }	0	0	52	K-IRS	{FATS,FFA,INS,PTPS}	0	0
8	K-IRS	{GLUCOSE }	0	0	53	K-IRS	{AKT,FATS,FFA,PTPS}	0	0
9	K-IRS	{INS}	0	0	54	K-IRS	{AKT,GLUCOSE,INS,PTPS}	0	0
10	K-IRS	{PTPS}	0	0	55	K-IRS	{AKT,FATS,FFA,GLUCOSE}	0	0
11	K-IRS	{AKT,FATS}	0	0	56	K-IRS	{FATS,GLUCOSE,INS,PTPS}	0	0
12	K-IRS	{FATS,FFA }	0	0	57	K-IRS	{AKT,FATS,GLUCOSE,INS}	0	0
13	K-IRS	{FATS,GLUOSE}	0	0	58	K-IRS	{AKT,FFA,GLUCOSE,INS}	0	0
14	K-IRS	{FATS,INS}	0	0	59	K-IRS	{AKT,FFA,GLUCOSE,PTPS}	0	0
15	K-IRS	{FATS,PTPS}	0	0	60	K-IRS	{AKT,FATS,GLUCOSE,PTPS}	0	0
16	K-IRS	{AKT,FFA}	0	0	61	K-IRS	{AKT,FATS,FFA,GLUCOSE,INS}	0	0
17	K-IRS	{FFA,GLUCOSE}	0	0	62	K-IRS	{AKT,FATS,FFA,INS,PTPS}	0	0
18	K-IRS	{FFA,INS}	0	0	63	K-IRS	{AKT,FFA,GLUCOSE,INS,PTPS}	0	0
19	K-IRS	{FFA,PPTS}	0	0	64	K-IRS	{FATS,FFA,GLUCOSE,INS,PTPS}	0	0
20	K-IRS	{AKT,GLUCOSE}	0	0	65	K-IRS	{AKT,FATS,GLUCOSE,INS,PTPS}	0	0
21	K-IRS	{GLUCOSE,INS}	0	0	66	K-IRS	{AKT,FATS,FFA,GLUCOSE,PTPS}	1	1
22	K-IRS	{GLUCOSE,PTPS}	0	0	67	K-IRS	{AKT,FATS,FFA,GLUCOSE,INS,PTPS}	1	1
23	K-IRS	{AKT,INS}	0	0	68	K_AKT	{}	0	0
24	K-IRS	{INS,PTPS}	0		69	K_AKT	{FATS}	0	0
25	K-IRS	{AKT,PTPS}	0	0	70	K_AKT	{FFA}	0	0
26	K-IRS	{AKT,FFA,INS}	0	0	71	K_AKT	{GLUCOSE}	0	0
27	K-IRS	{FATS,GLUCOSE,INS}	0	0	72	K_AKT	{IRS}	0,1	
28	K-IRS	{FATS,GLUCOSE,PTPS}	0	0	73	K_AKT	{FATS,FFA}	0	0
29	K-IRS	{AKT,FFA,PTPS}	0	0	74	K_AKT	{FFA,GLUCOSE}	0	0
30	K-IRS	{FATS,INS,PTPS}	0	0	75	K_AKT	{FFA,IRS}	0,1	0
31	K-IRS	{AKT,FATS,GLUCOSE}	0	0	76	K_AKT	{FATS,GLUCOSE}	0	0
32	K-IRS	{AKT,GLUCOSE.INS}	0	0	77	K_AKT	{GLUCOSE,IRS}	0,1	0
33	K-IRS	{AKT,GLUCOSE,PTPS}	0	0	78	K_AKT	{FATS,IRS}	0,1	0
34	K-IRS	{FFA,GLUCOSE,INS}	0	0	79	K_AKT	{FATS,FFA,IRS}	0,1	0
35	K-IRS	{FFA,GLUCOSE,PTPS}	0	0	80	K_AKT	{FATS,FFA,GLUCOSE}	1	1
36	K-IRS	{AKT,FATS,INS}	0	0	81	K_AKT	{FATS,GLUCOSE,IRS}	0,1	0
37	K-IRS	{FFA,INS,PTPS}	0	0	82	K_AKT	{FFA,GLUCOSE,IRS}	0,1	0
38	K-IRS	{AKT,INS,PTPS}	0	0	83	K_AKT	{FATS,FFA,GLUCOSE,IRS}	1	1
39	K-IRS	{AKT,FATS,PTPS}	0	0	84	K_FATS	{}	1	1
40	K-IRS	{AKT,FATS,FFA}	0	0	85	K_GLUCOSE	{}	1	1
41	K-IRS	{GLUCOSE,INS,PTPS}	0	0	86	K_CPTB	{}	0	0
42	K-IRS	{AKT,FFA,GLUCOSE}	0	0	87	K_CPTB	{FATS}	1	1
43	K-IRS	{FATS,FFA,GLUCOSE}	0	0	88	K_CPTB	{FFA}	0,1	0
44	K-IRS	{FATS,FFA,INS}	0	0	89	K_CPTB	{FATS,FFA}	1	1
45	K-IRS	{FATS,FFA,PTPS}	0	0	90	K_FFA	{}	1	1

as "1", as well as "0" indicates the expression level the entity that is below the activation threshold or in inactive mode.

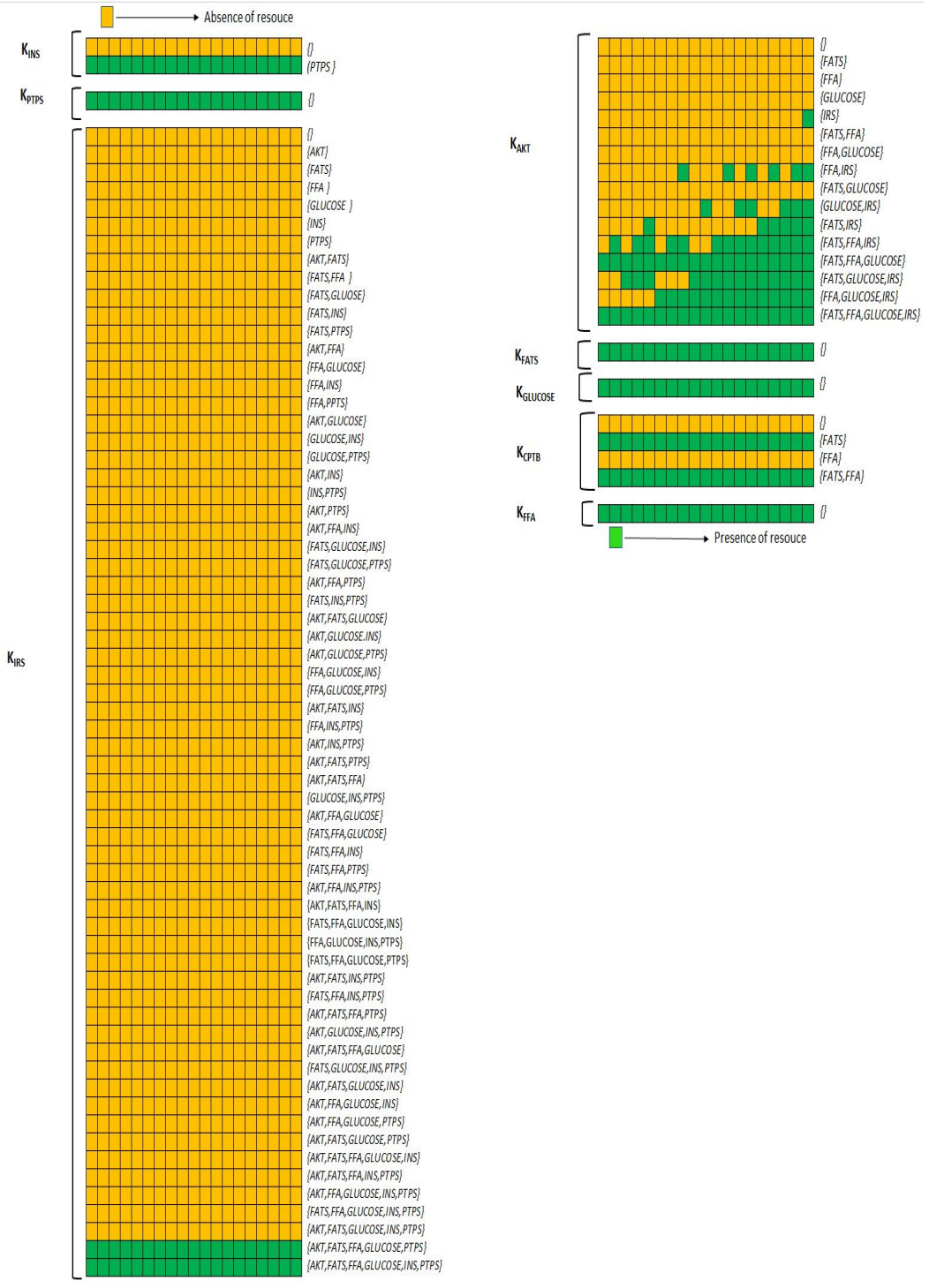


FIGURE 4.6: Heat-map. The expression is represented with green whereas the down-regulated values are shown as orange rectangles

4.6 Hybrid Modeling Results

Hybrid modeling had been completed while using HyTech (HYbrid TECHnology) . The delay constraints in the qualitative condition (1, 0, 0, 0, 0, 0, 0, 0) towards the deadlock condition (0, 0, 0, 1, 1, 1, 1, 0) tend to be computed and which highlight important relations between the production and degradation rates of important proteins. The results show that absolute value of the degradation delay of dnINS1 <= dnAKT1 and production delay of dpOBESITY0 <= dpFFA0 & dpPTPS0 <= dpOBESITY0.

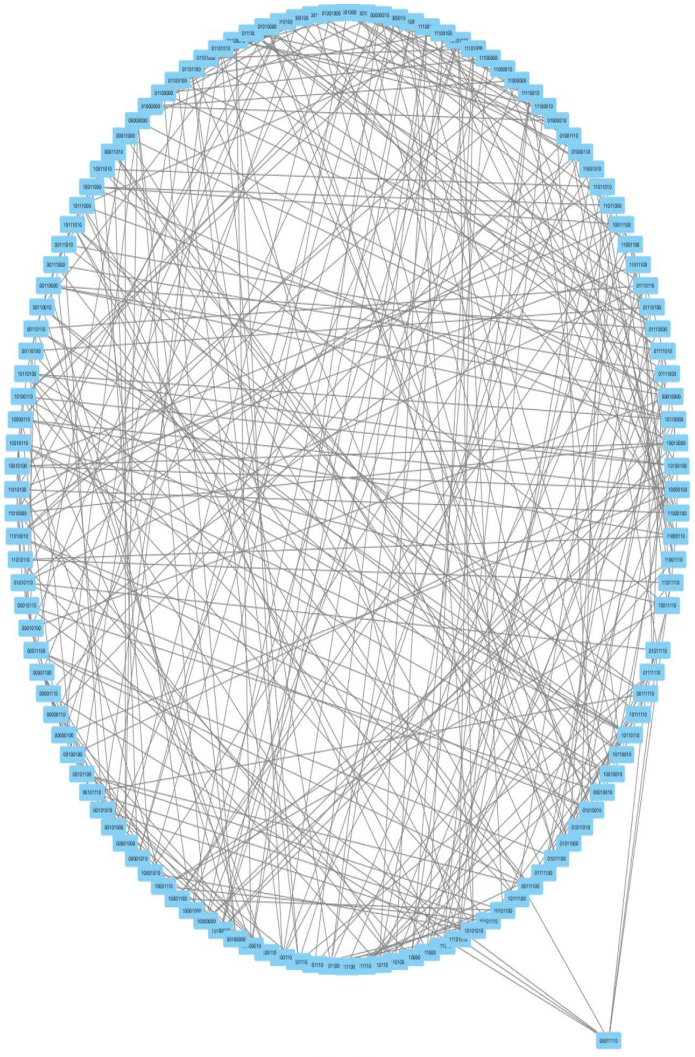


FIGURE 4.7: Qualitative state graph.

This result shows that INS is degraded earlier then AKT because its time delayed is less than AKT. On the other hand we have production delays that

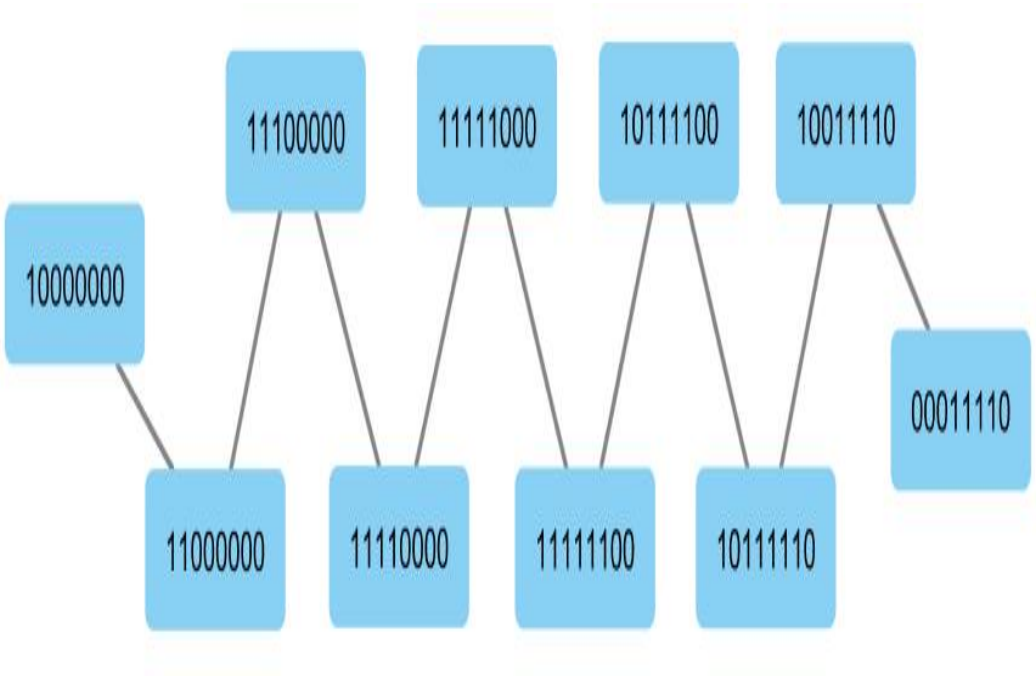


FIGURE 4.8: Sub graph extracted from the state transition graph.

show us PTPS is produced earlier then OBESITY and OBESITY is produced earlier then FFA. This shows that PTPS will start inhibiting the INS receptor in the system much faster then OBESITY because PTPS is produced earlier then OBESITY.

4.7 Stochastic Petri Net Modeling Results

As mentioned within Chapter2 of the thesis, biological relationships tend to be stochastic within nature therefore we use a stochastic Petri net modeling. Figure 4.9 shows the stochastic Petri net model of Insulin resistance Pathway. The results generated of this model are in the form of graphs that fully satisfy the results of hybrid modeling.

Figure 4.10 shows the results of Petri net modeling. The Petri net model had been run (in Snoopy software) by using Gillespie's algorithm. The stimulation trajectories tend to be proven is shown in figure 4.10 along with the expression on y-axis plotted against time on x-axis.

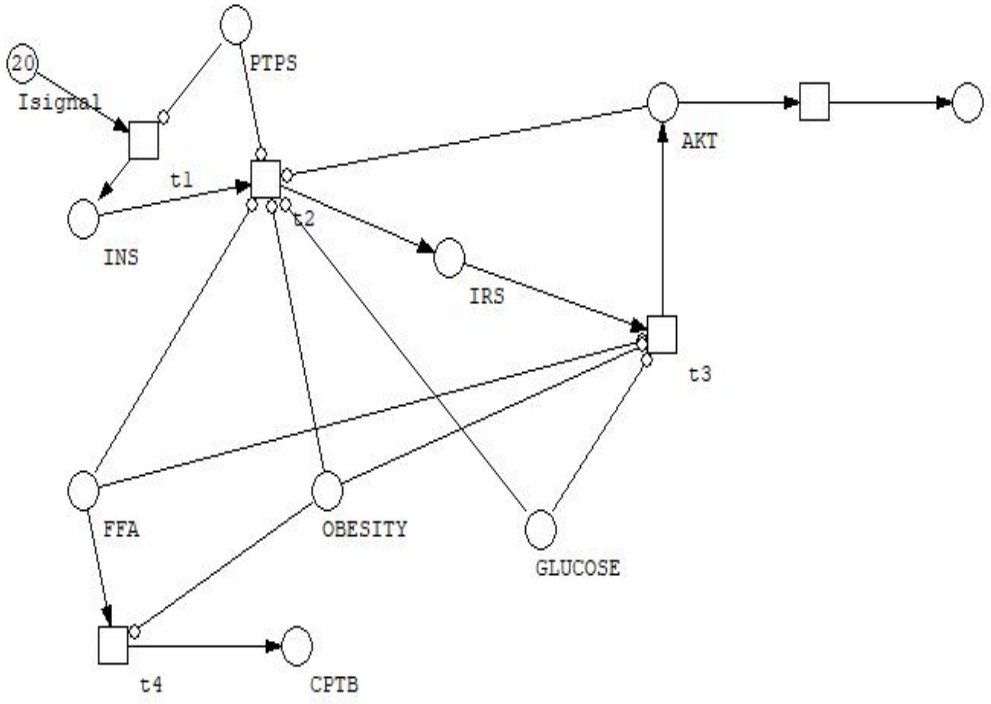


FIGURE 4.9: Stochastic-petriNet model of Insulin resistance.

Diabetes mellitus is currently thought to be the particular world's most common metabolic problem [70]. Based on current epidemiological research, United States has got the greatest frequency associated with diabetes on the planet, along with 21.4 million individuals, or even 7.8 percent from the adult population [71]. The deregulation associated with insulin secretion or even signaling with the IR results in development within diabetes [72].

One essential mechanism within controlling insulin signaling is mediated through PTPs, which might ether act on the IR by itself or even it's substrates [72].Among the very first experience related to PTPs within insulin signaling as well as diabetes originated from earlier research utilizing vanadium which normalized blood sugar amounts within diabetic animal models [73,74]. Through all of the PTPs present in insulin-sensitive tissue, numerous laboratories provided biochemical proof associated with PTPs as a critical regulator of the IR signaling path through inhibiting insulin signaling. Types of possible candidates consist of receptors PTPalpha as well as PTPepsilon [75].

A number of research analyzed PTP1B activity within rats as well as humans along with insulin resistance, diabetes, as well as being overweight. Numerous reviews demonstrated elevated activity associated with PTP1B within these types. Within skeletal muscle associated with insulin-resistant overweight (fa/fa) as well as Zucker diabetic fatty (ZDFfa/fa) rodents, a rise within vitro dephosphorylation associated with IR correlating along with greater PTP activity had been noticed [66].

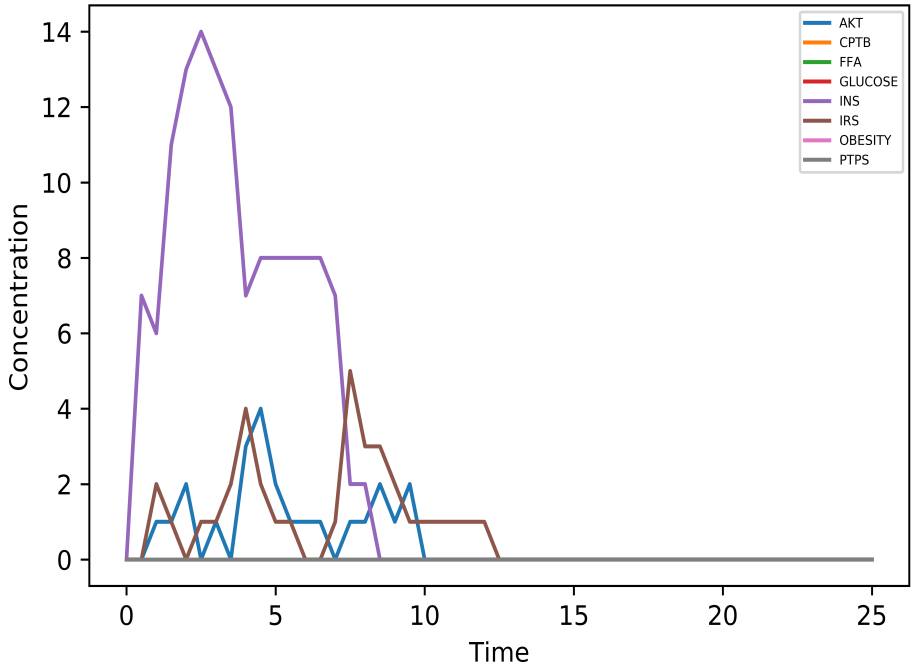


FIGURE 4.10: Result of Petri net modeling.

Rise within PTP1B activity as well as expression had been additionally seen in skeletal muscle tissue taken from non-obese, Goto -Kakizaki insulin resistant type 2 diabetic (GK) rodents. Insulin-stimulated IR autophosphorylation as well as IRS1 tyrosine phosphorylation had been inhibited within GK skeletal muscle tissue when compared with non-diabetic rodents, suggesting that increased PTP1B activity can lead to rise in sugar level as well as insulin resistance within GK rodents [67].Additionally, it had been shown that PTP1B proteins level is actually increased within fructose-fed hamster model of insulin resistant.[68]. Fat

loss and also development regarding insulin level of sensitivity inside human subjects correlate together with lowered PTP action [69].

Chapter 5

Conclusion and Future Recommendations

With this we now have proven that qualitative as well as quantitative modeling reveal different factors associated with signaling paths. With the help of Qualitative modeling we examined the step wise working of Insulin resistance pathway from starting point to its stable state. We selected the most important route based on typical betweenness centrality through analyzing just about all pathways from the provided qualitative condition towards the target state. The order of entities present in each state is INS, IRS, AKT, PTPS, OBESITY, FFA, GLUCOSE, CPTB. After getting a state graph we used it in Hybrid modeling that gives us results of activation and degradation of important entities with respect to time that AKT is degraded earlier then IRS because its time delayed is less than IRS and INS is degraded earlier then AKT because its time delayed is less than AKT. On the other hand we have production delays that show us PTPS is produced earlier then OBESITY and OBESITY is produced earlier then FFA. This shows us the relationship that we have two entities that are produced earlier and those two entities inhibit the INS and AKT which has the degradation earlier then others. With the help of quantitative modeling we have confirmatory results that INS is degraded earlier then IRS and AKT. All these results show that while targeting Diabetes we should look for OBESITY and PTPS also. These two are the crucial

that disturb the normal pathway of Insulin signaling in muscles.

Bibliography

[1] Entmacher PS, Marks HH. Diabetes in 1964; a world survey. Diabetes, 14:212–23, 1965.

[2] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 27:1047–53, 2004.

[3] International Diabetes Federation. IDF Diabetes Atlas. 1st ed. Brussels, Belgium: International Diabetes Federation; 2000(04/27/2020).

[4] International Diabetes Federation. IDF Diabetes Atlas. 2nd ed. Brussels, Belgium: International Diabetes Federation; 2003 (04/27/2020).

[5] International Diabetes Federation. IDF Diabetes Atlas. 3rd ed. Brussels, Belgium: International Diabetes Federation; 2006 (04/27/2020).

[6] International Diabetes Federation. IDF Diabetes Atlas. 4th ed. Brussels, Belgium: International Diabetes Federation; 2009. (04/27/2020).

[7] International Diabetes Federation. IDF Diabetes Atlas. 5th ed. Brussels, Belgium: International Diabetes Federation; 2011. (04/27/2020).

[8] International Diabetes Federation. IDF Diabetes atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2013. (04/27/2020).

[9] World Health Organization. Health in 2015, from MDGs, millennium development goals to SDGs, sustainable development goals; 2015.

[10] Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., ... & Makaroff, L. E. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice, 128, 40-50, 2017.

[11] Voet, D., & Voet, J. G. Biochemistry, 4-th Edition. NewYork: John Wiley& SonsInc, 492-496, 2011.

[12] Bliss, M. The history of insulin. Diabetes Care, 16(Supplement 3), 4-7, 1993.

[13] Alberti, K. G. M. M., Zimmet, P., & DeFronzo, R. A. International textbook of diabetes mellitus. J. Wiley. 1997

[14] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care, 26:5-20, 2003. (04/27/2020).

[15] Mokdad A H , Ford E S , Bowman B A , Dietz W H , Vinicor F , Bales V S , Marks J S . Prevalence of obesity, diabetes, and obesity-related health risk factors, JAMA, 289(1):76–79, 2001.

[16] Ginsberg, H. E. N. R. Y., Kimmerling, G. E. O. R. G. E., Olefsky, J. M., & Reaven, G. M. Demonstration of insulin resistance in untreated adult onset diabetic subjects with fasting hyperglycemia. The Journal of clinical investigation, 55(3), 454-461, 1975.

[17] Roth, J. Peptide hormone binding to receptors: a review of direct studies in vitro. Metabolism, 22(8), 1059-1073, 1973.

[18] Kolterman, O. G., Insel, J., Saekow, M., & Olefsky, J. M. Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects. The Journal of clinical investigation, 65(6), 1272-1284, 1980.

[19] Hotamisligil, GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. J Intern Med, 245:621-625, 1999.

[20] Friedman, JM. Obesity in the new millennium. Nature, 404:632-634, 2000.

[21] Goldstein, BJ, Ahmad, F, Ding, W, Li, PM, Zhang, WR. Regulation of the insulin signalling pathway by cellular protein-tyrosine phosphatases. Mol Cell Biochem, 182:91-99, 1998.

[22] Klaman, LD, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B (PTP1B)-deficient mice. Mol Cell Biol, 20:5479-5489, 2000.

[23] Bj orn H. Juncker and Falk Schreiber. Analysis of Biological Networks. John Wiley & Sons, Inc., Hoboken, NJ, USA, 2008.

[24] Rene Thomas and Richard d'Ari. Biological feedback. CRC press, 1990.

[25] Jamil Ahmad, Gilles Bernot, J-P Comet, Didier Lime, and Olivier Roux. Hybrid modelling and dynamical analysis of gene regulatory networks with delays. ComPlexUs, 3(4):231–251, 2007.

[26] Pavlopoulos GA, Secrier M, Moschopoulos CN, Soldatos TG, Kossida S, et al. Using graph theory to analyze biological networks. BioData mining, 4: 10, 2011.

[27] Barabasi AL, Oltvai ZN Network biology: understanding the cell's functional organization. Nature Reviews Genetics, 5: 101–113, 2004.

[28] Mason O, Verwoerd M Graph theory and networks in biology. IET systems biology, 1: 89–119, 2007.

[29] Aittokallio T, Schwikowski B Graph-based methods for analysing networks in cell biology. Briefings in bioinformatics, 7: 243–255, 2006.

[30] Wasserman S Social network analysis: Methods and applications, volume 8. Cambridge university press, 1994.

[31] Koschützki D, Schreiber F Centrality analysis methods for biological networks and their application to gene regulatory networks. Gene regulation and systems biology 2: 193, 2008.

[32] Ahmad J, Bernot G, Comet JP, Lime D, Roux O Hybrid modeling and dynamical analysis of gene regulatory networks with delays. ComPlex Us, 3: 231–251, 2007.

[33] Aslam B, Ahmad J, Ali A, Zafar Paracha R, Tareen SHK, et al. On the modelling and analysis of the regulatory network of dengue virus pathogenesis and clearance. Computational Biology and Chemistry, 53: 277–291, 2014.

[34] Naldi A, Remy E, Thieffry D, Chaouiya C. A reduction of logical regulatory graphs preserving essential dynamical properties. In: International Conference on Computational Methods in Systems Biology. Springer, pp. 266–280, 2009.

[35] Saadat pour A, Albert R, Reluga TC. A reduction method for Boolean network models proven to conserve attractors. SIAM Journal on Applied Dynamical Systems, 12: 1997–2011, 2013.

[36] D Kartson, Gianfranco Balbo, S Donatelli, G Franceschinis, and Giuseppe Conte. Modeling with generalized stochastic Petri nets. John Wiley & Sons, Inc., 1994.

[37] Bernot G, Comet JP, Richard A, Guespin J. Application of formal methods to biological regulatory networks: extending Thomas' asynchronous logical approach with temporal logic. Journal of theoretical biology, 229: 339–347, 2004.

[38] Clarke EM, Grumberg O, Peled D. Model checking. MIT press, 1999.

[39] Marsan MA. Stochastic petri nets: an elementary introduction. In: European Workshop on Applications and Theory in Petri Nets. Springer, pp. 1–29, 1988.

[40] Sakamoto K, Holman GD: Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. Am J Physiol Endocrinol Metab, 295: 29-37, 2008.

[41] Shulman GI: Cellular mechanisms of insulin resistance. J Clin Invest, 106: 171-176, 2000.

[42] Roden M: How free fatty acids inhibit glucose utilization in human skeletal muscle. News Physiol Sci, 19: 92-96 2004.

[43] Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI: Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes, 48: 1270-1274, 1999.

[44] Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, Shulman GI: Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem, 277: 50230-50236, 2002.

[45] Randle PJ: Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev, 14: 263-283, 1998.

[46] Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest, 103: 253-259, 1999.

[47] Schmitz-Peiffer C: Signalling aspects of insulin resistance in skeletal muscle: mechanisms induced by lipid oversupply. Cell Signal, 12: 583-594, 2000.

[48] Cooney GJ, Thompson AL, Furler SM, Ye J, Kraegen EW: Muscle long-chain acyl CoA esters and insulin resistance. Ann N Y Acad Sci, 967: 196-207, 2002.

[49] Itani SI, Ruderman NB, Schmieder F, Boden G: Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes, 51: 2005-2011, 2002.

[50] Summers SA: Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res, 45: 42-72, 2006.

[51] Nagle CA, Klett EL, Coleman RA: Hepatic triacylglycerol accumulation and insulin resistance. J Lipid Res, 50: S74-S79, 2009.

[52] De Jong H. Modeling and simulation of genetic regulatory systems: a literature review. Journal of computational biology, 9: 67–103, 2002.

[53] Sachs R, Hlatky L, Hahnfeldt P. Simple ode models of tumor growth and anti-angiogenic or radiation treatment. Mathematical and Computer Modeling, 33: 1297–1305, 2001.

[54] Diagnosis and classification of diabetes mellitus.American Diabetes Association Diabetes Care, 37 Suppl 1():S81-90, jan 2014.

[55] Diagnosis and classification of autoimmune diabetes mellitus. Canivell S, Gomis R Autoimmun Rev, 13(4-5):403-7, Apr-May 2014.

[56] Beal, M.J., Falciani, F., Ghahramani, Z., Rangel, C. and Wild, D.L. A Bayesian approach to reconstructing genetic regulatory networks with hidden factors. Bioin- formatics, 21, 349-356 2005.

[57] Armaanzas, R., Inza, I. and Larraaga, P. Computer Methods and Programs in Biomedicine, 91, 110-121 2008.

[58] Maraziotis, Ioannis A., Andrei Dragomir, and Dimitris Thanos. "Gene regulatory networks modelling using a dynamic evolutionary hybrid." BMC bioinformatics 11, no. 1, 140, 2010.

[59] Bae, Kiho, Choogon Lee, Paul E. Hardin, and Isaac Edery. "dCLOCK is present in limiting amounts and likely mediates daily interactions between the dCLOCK–CYC transcription factor and the PER–TIM complex." Journal of Neuroscience20, no. 5, 1746-1753, 2000.

[60] Chen, Ting, Hongyu L. He, and George M. Church. "Modeling gene expression with differential equations." In Biocomputing'99, pp. 29-40. 1999.

[61] Vladimir, F. Handbook of computational molecular biology. University of California, Davis, 2005.

[62] Shmulevich, Ilya, Ilya Gluhovsky, Ronaldo F. Hashimoto, Edward R. Dougherty, and Wei Zhang. "Steady-state analysis of genetic regulatory networks modelled by probabilistic Boolean networks." Comparative and functional genomics 4, no. 6, 601-608, 2003.

[63] Fauré, Adrien, Aurélien Naldi, Claudine Chaouiya, and Denis Thieffry. "Dynamical analysis of a generic Boolean model for the control of the mammalian cell cycle." Bioinformatics 22, no. 14, e124-e131, 2006.

[64] Gagniuc, Paul A. Markov Chains: From Theory to Implementation and Experimentation. USA, NJ: John Wiley & Sons. pp. 46–59, 2017.

[65] Feliu, E., & Wiuf, C. Finding the positive feedback loops underlying multistationarity. BMC systems biology, 9(1), 22, 2015.

[66] F. Ahmad, B.J. Goldstein, Increased abundance of specific skeletal muscle protein –tyrosine phosphatases in a genetic model of insulinresistant obesity and diabetes mellitus, Metabolism 44, 1175 – 1184, 1995.

[67] S.S. Dadke, H.C. Li, A.B. Kusari, N. Begum, J. Kusari, Elevated expression and activity of protein –tyrosine phosphatase 1B in skeletal muscle of insulinresistant type II diabetic Goto – Kakizaki rats, Biochem. Biophys. Res. Commun. 274, 583 – 589, 2000.

[68] C. Taghibiglou, F. Rashid-Kolvear, S.C. Van Iderstine, H. Le-Tien, I.G. Fantus, G.F. Lewis, K. Adeli, Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein –tyrosine phosphatase 1B in a fructose-fed hamster model of insulin resistance, J. Biol. Chem. 277, 793 – 803, 2002.

[69] F. Ahmad, R.V. Considine, T.L. Bauer, J.P. Ohannesian, C.C. Marco, B.J. Goldstein, Improved sensitivity to insulin in obese subjects following weight

loss is accompanied by reduced protein –tyrosine phosphatases in adipose tissue, Metabolism 46, 1140 – 1145, 1997.

[70] A.R. Saltiel, New perspectives into the molecular pathogenesis and treatment of type 2 diabetes, Cell 104, 517 – 529, 2001.

[71] S. Wild, G. Roglic, A. Green, R. Sicree, H. King, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030, Diabetes Care 27, 1047 – 1053, 2004.

[72] A. Cheng, N. Dube, F. Gu, M.L. Tremblay, Coordinated action of protein tyrosine phosphatases in insulin signal transduction, Eur. J. Biochem. 269, 1050 – 1059, 2002.

[73] I.G. Fantus, G. Deragon, R. Lai, S. Tang, Modulation of insulin action by vanadate: evidence of a role for phosphotyrosine phosphatase activity to alter cellular signaling, Mol. Cell. Biochem. 153, 103 – 112, 1995.

[74] I.G. Fantus, E. Tsiani, Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects, Mol. Cell. Biochem. 182,109 – 119, 1998.

[75] N.P. Moller, K.B. Moller, R. Lammers, A. Kharitonenkov, E. Hoppe, F.C. Wiberg, I. Sures, A. Ullrich, Selective down-regulation of the insulin receptor signal by protein –tyrosine phosphatases alpha and epsilon, J. Biol. Chem. 270, 23126 – 23131, 1995.

[76] Hedley, A. A. et al. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. J. Am. Med. Assoc. 291, 2847–2850 2004.

[77] World Health Organization Consultation on Obesity 1–253 (World Health Organization, Geneva, 2000).

[78] Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27, 1047–1053 2004.

[79] Reaven, G. M. Role of insulin resistance in human disease. Diabetes 37, 1595–1607 1988.

[80] Perley, M. and Kipnis, D. M. Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes15, 867–874 1966.

[81] Polonsky, K. S., Given, B. D. and Van Cauter, E. Twenty-four-hour profiles and patterns of insulin secretion in normal and obese subjects. J. Clin. Invest. 81, 442–448 1988.

[82] Kahn, S. E. et al. Quantification of the relationship between insulin sensitivity and B-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 42, 1663–1672 1993.

[83] Kahn, S. E. The importance of Beta-cell failure in the development and progression of type 2 diabetes. J. Clin. Endocrinol. Metab. 86, 4047–4058 2001.

[84] Moran, A. et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 48, 2039–2044 1999.

[85] Buchanan, T. A., Metzger, B. E., Freinkel, N. and Bergman, R. N. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. Am. J. Obstet. Gynecol.162, 1008–1014 1990.

[86] DeFronzo, R. A. Glucose intolerance of aging. Evidence for tissue insensitivity to insulin. Diabetes 28, 1095–1101 1979.

[87] Goodyear, L. J. and Kahn, B. B. Exercise, glucose transport, and insulin sensitivity. Annu. Rev. Med. 49, 235–261 1998.

[88] Chen, M., Bergman, R. N. and Porte, D. Insulin resistance and Beta-cell dysfunction in aging: the importance of dietary carbohydrate. J. Clin. Endocrinol. Metab. 67, 951–957 1988.

[89] Wellen, K. E. and Hotamisligil, G. S. Inflammation, stress, and diabetes. J. Clin. Invest.115, 1111–1119 2005.

[90] Scherer, P. E. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55, 1537–1545 2006.

[91] Shoelson, S. E., Lee, J. and Goldfine, A. B. Inflammation and insulin resistance. J. Clin. Invest. 116, 1793–1801 2006.

[92] Yang, Q. et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436, 356–362 2005.

[93] Kadowaki, T. et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Invest.116, 1784–1792 2006.

[94] Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P. and Bahouth, S. W. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 145, 2273–2282 2004.

[95] Reaven, G. M., Hollenbeck, C., Jeng, C. Y., Wu, M. S. and Chen, Y. D. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes 37, 1020–1024 1988.

[96] Boden, G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46, 3–10 1997.

[97] Roden, M. et al. Mechanism of free fatty acid-induced insulin resistance in humans. J. Clin. Invest. 97, 2859–2865 1996.

[98] Santomauro, A. T. et al. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes 48, 1836–1841 1999.

[99] Randle, P. J., Garland, P. B., Hales, C. N. and Newsholme, E. A. The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet i, 785–789 1963.

[100] Shulman, G. I. Cellular mechanisms of insulin resistance. J. Clin. Invest.106, 171–176 2000.

[101] White MF. The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. Recent Prog Horm Res. 53, 119-138, 1998.

[102] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7, 85-96, 2006.

[103] Sale EM, Sale GJ. Protein kinase B: signalling roles and therapeutic targeting. Cell Mol Life Sci 65, 113-127, 2008.

[104] Farese RV, Sajan MP, Standaert ML. Insulin-sensitive protein kinases (atypical protein kinase C and protein kinase B/Akt): actions and defects in obesity and type II diabetes. Exp Biol Me(Maywood). 230, 593-605, 2005.

[105] Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of Obesity, Diabetes, and Obesity-Related Health Risk Factors, 2001. JAMA.289(1),76–79, 2003

[106] Mokdad AH, Bowman BA, Ford ES, Vinicoor F, Marks JS et al.,. The continuing epidemics of obesity and diabetes in the United States. JAMA. 286, 1195-1200, 2001.

[107] Venkataramana R, Nanda NC, Baweja G, Parikh N, Bhatia V. Prevalence of diabetes mellitus and related conditions in Asian Indians living in the United States. Am J Cardiol. l, 94, 977-980, 2004.

[108] Herder C, Roden M. "Genetics of type2 diabetes: pathophysiologic and clinical relevance". European Journal of clinical investigation. 41(6), 679-92, 2011

[109] Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring study. Diabetes. 49(12), 2201-7, 2000

[110] Doria A, Patti ME, Kahn CR. The emerging genetic architechture of type 2 diabetes. Cell Metab. 8(3), 186-200, 2008