Preprints of the 12th IFAC Symposium on Computer Applications in Biotechnology
The International Federation of Automatic Control
16-18, 2013, December. Mumbai, India

# Analysis of Integrated Insulin-mTOR Signalling Network -Diabetes Perspective

**Pramod R. Somvanshi*, Anilkumar K. Patel*, Sharad Bhartiya*, K.V.Venkatesh***

** Departmentof Chemical Engineering, Indian Institute of Technology, Bombay, Mumbai, INDIA. (email-pramod.rs@iitb.ac.in, venks@iitb.ac.in)*

Abstract: The regulatory action of insulin on the blood glucose homeostasis is mediated by the Insulin signalling pathway. To quantify this regulation, we have integrated the models of Insulin secretion, insulin signalling, mTOR signalling and blood glucose uptake by various tissues. We have analyzed the effect of perturbations in the integrated insulin-mTOR signalling pathway on blood glucose levels. These effects were studied in the tissues in which glucose uptake is dependent on insulin (i.e. muscle, adipose and heart). Seventy five rate parameters in the integrated network were perturbed and the corresponding steady state plasma glucose levels were recorded. The fold changes in these parameters leading to pre-diabetic and diabetic states were characterized. Perturbations in 22 parameters elicited diabetic effect and 31 parameters showed pre-diabetic effects for certain fold change in basal parameter values. In the insulin signalling pathway, the concentrations and phosphorylation states of PTP and PIP3 were most effective in leading to diabetic state with only 3 and -3 fold change in their parameters, respectively. While increasing concentrations of PTEN by five folds also led to diabetic state, decreasing the phosphorylation of IRS, AKT and GSK3 unto 10 folds resulted in diabetic state. In the mTOR pathway, increasing the influence of amino acid on Rheb-GTP localization to the inhibitory complex by 4 folds elicited diabetic state. On the other hand decreasing conversion of RhebGTP to RhebGDP and formation of mTOR-raptor-PRAS40 complex also resulted in diabetic state. This demonstrates the influence of excess amino acid intake on diabetic state. Overall, it was noted that only 2 to 3 fold change in sensitive parameters resulted in pre-diabetic state.

*Keywords:* Insulin signalling pathway, glucose homeostasis, mTOR pathway, perturbation analysis, Diabetes, Impaired Fasting Glucose.

## 1.INTRODUCTION

Insulin Resistance is the key component of diabetic pathology. Insulin is the major anabolic regulatory hormone involved in growth, differentiation and metabolism. The metabolic homeostasis is regulated in coordination with the differential effects of insulin on various kinds of tissues. The glucose homeostasis in blood is maintained through insulin by stimulating glucose uptake, utilization and storage in muscle and adipose tissues, whereas in liver insulin suppresses hepatic gluconeogenesis by promoting glycogen synthesis. The regulatory effects of insulin are mediated by various signalling events to the downstream metabolic effectors. This pathway is central to metabolic regulations and has crosstalk with various other pathways for coordinated control of cellular events. The inability of the cellular environment to respond to the circulating levels of insulin is characterized by insulin resistance (Saltiel et al., 2001). Research has shown that the defects in insulin secretion, Insulin signalling and Insulin action can lead to insulin resistance leading to diabetes (Sesti 2006; Leng et al., 2004).

Cells are constantly subjected to the varying environment cues and respond to the stimulus based on the underlying design of the structure. The burden of unhealthy diet and lifestyle can impose the cells to drastic environment which in turn can lead to changes in the cellular response due to alteration in some of the baseline parameters. Biologically such changes in parameters can occur due to over expression or under expression of respective genes, activation or inhibition due to other metabolites, crosstalk between pathways and due to altered strength of feedback mechanisms. Any modification to robust setting either due to over activation of the positive or negative feedback can result in a disease state (Kitano, 2004).

To quantify the contribution of the signalling pathway to the insulin resistance, it is essential to integrate the models of insulin signalling pathway and its crosstalk and feedback regulation with other metabolic pathway. Moreover, to analyze the effect of these pathways on plasma glucose levels and its feedback on insulin secretion, the module of insulin secretion and glucose uptake by different tissues need to be connected with appropriate mathematical representation. Here we have integrated the models for insulin signalling (Sedaghat et al., 2002), mTOR signalling (Vinod et al., 2009), Insulin secretion by pancreas (Dalla Man 2007) and glucose uptake and performed purterbation analysis to characterize the parameters that lead to diabetic state.

## 2. INTEGRATED SIGNALLING NETWORK

The metabolic insulin signalling pathway is one of the central pathways in metabolic regulation of carbohydrates, fats and proteins. Various signalling intermediates have been known to

elicit regulatory properties which help in system level management of the cellular metabolic and energy status (Kahn, 2002). The integrated insulin-mTOR signalling network is shown in Fig.1. Insulin binds to the insulin receptor leading to its autophosphorylaton and subsequent tyrosine phosphorylation of IRS1. Phosphorylation of IRS1 activates PI3K, which in turn catalyzes production of PIP2 and PIP3. These phosphoinositol lipids activate PDK1 leading to the phosphorylation of downstream protein kinases such as AKT (PKB) and PKC which coordinate the translocation of intracellular GLUT4 vesicles to the plasma membrane. Moreover AKT phosphorylates active GSK3 which suppress Glycogen synthase. The inactivation of GSK3 facilitates glycogen synthesis via insulin mediated AKT action (Sedaghat et al., 2002; Giri et al., 2004).

Activation of mTOR pathway is essential in protein synthesis which is regulated by insulin and amino acids. Insulin regulates activation of mTOR through the action of AKT. AKT inhibits the formation of tuberous sclerosis heterodimer by phosphorylation of TSC that inactivates Rheb by conversion of RhebGTP to RhebGDP. Rheb-GTP activates mTORC1 by freeing it from the mTOR-raptor-FKBP38 complex by the removal of the inhibitor FKBP38 leading to formation of FKBP38-RhebGTP complex. Thereafter, mTOR-raptor complex phosphorylates S6K1 and activates it for protein synthesis. Moreover, mTOR exist in two forms, mTORC1 and mTORC2 complex. mTORC2 (mTOR-rictor complex) is activated by PI3 kinase and amino acids. mTORC2 further activates AKT by phosphorylation. Amino acids have multiple site regulation mTOR pathways ((Virgilio and Loewith, 2006; Vinod et al., 2009).

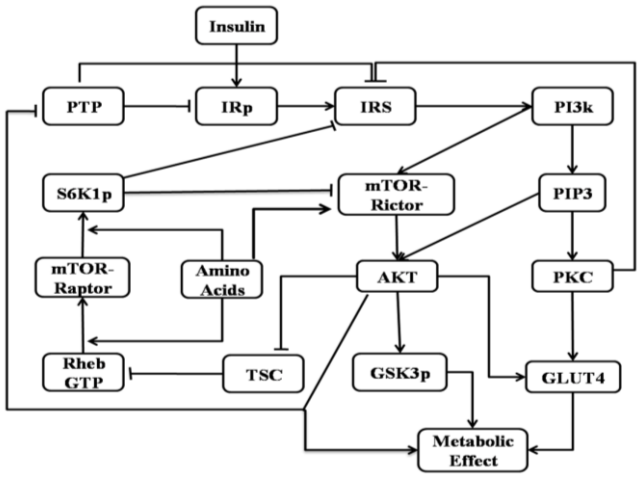



Figure.1) Integrated insulin-mTOR signalling network.

Insulin signalling pathway is subjected to multiple feedback regulation. PTP negatively regulates the insulin signalling by dephosphorylating insulin receptor and IRS1 thereby attenuating its action. Whereas AKT inactivates phosphatase PTP leading to a positive feedback loop in the pathway. Negative feedback regulation involves activation of PKC-ζ, which phosphorylate serine residues in IRS1 to prevent tyrosine phosphorylation of IRS1. Furthermore, mTOR signalling activates S6K1 which exerts a negative feedback on insulin signalling by serine phosphorylation of IRS1 and inhibition of formation of mTORC-rictor complex which in turn activates AKT (Giri et al., 2004; Vinod, 2009).

## 3. MODEL DESCRIPTION

The modular approach was adopted to analyze this system. The model components required for this analysis were selected from the literature and subsequently integrated by connecting the output of one module as the input to the other module. The model was divided into three modules, namely 1) Insulin kinetics Module- dealing with Insulin secretion and its kinetics; 2) Glucose Kinetics Module- dealing with plasma glucose kinetics; 3) Signalling Kinetics Module-dealing with Integrated Insulin-mTOR signalling pathway. The overall integrated model contains 46 ODEs, 48 rate equations and 112 parameters. The overall integrated model framework is as shown in Fig.2.

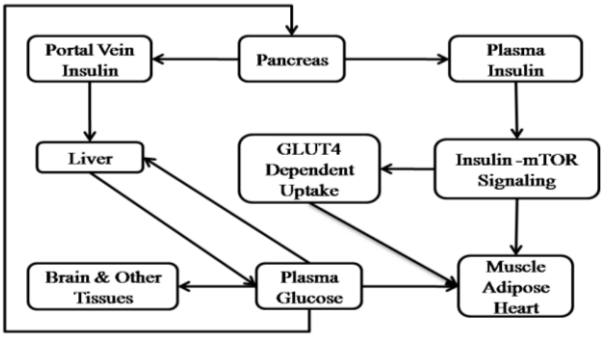



Figure.2) Schematic of the Integrated model of insulin-Glucose- Signalling Kinetics.

### *3.1. Insulin kinetics Module*

Insulin is secreted by the pancreatic β-cells in response to varying plasma glucose levels. Appropriate glucose sensing and insulin secretion by pancreas is essential to maintain plasma glucose levels in the narrow range of homeostatic levels around 4.5 to 5.5 mmol/l. We have referred to the model developed by Dalla Man et.al (2007, 2009) for the Insulin secretion by pancreas and insulin kinetics. The model describes the kinetics of insulin secretion by pancreases in response to the plasma glucose levels. In the current model formulation, the equations and parameter values for β-cell insulin secretion, portal vein insulin concentration, plasma and hepatic insulin concentrations were adopted from the model developed by Dalla Man (2007).

### *3.2.Glucose Kinetics Module*

Glucose homeostasis is one of the most tightly regulated phenomena in human physiology. It is regulated by the uptake, utilization and storage of excess plasma glucose (during postprandial state) by various tissues, and release of glucose in the blood by liver, during fasting and starvation. In the postprandial state plasma glucose level rises up to 8 to 10 mmol/l. During this state almost all the tissues uptake glucose in either insulin dependent manner or insulin independent manner to maintain the plasma glucose level around 5 mmol/l.

The excess glucose is stored as glycogen and fat in the tissues and some part is used as fuel for energy production. However, during fasting and starvation liver produces glucose by glycogen breakdown and gluconeogenesis. The rate of endogeneous glucose production by liver is almost equal to the net whole body glucose utilization rate. The postprandial regulation of glucose metabolism is mediated by the insulin. The glucose uptake in muscle, adipose and heart tissues are insulin dependent, which is facilitated by the translocation and activation of GLUT4 (glucose transporters) via insulin signalling pathway (Liu et al., 2008; Chew et al., 2009). The intracellular utilization of glucose is also regulated by insulin signalling intermediates such as AKT and GSK3. While AKT facilitates activation of glycolysis and lipogenesis, GSK3 regulates glycogen synthesis in the cells (Saltiel et al., 2001). The liver glucose metabolism is regulated by portal vein insulin and liver insulin. However, the glucose uptake by liver and kidney is non insulin dependent and is facilitated by GLUT2. In brain, erythrocytes, GI track and other tissues uptake glucose in non-insulin dependent manner, which is either facilitated by GLUT1 and GLUT3 or SGLT1 and SGLT2 (Sodium glucose co-transporters) (Frayn, 2010).

To model the glucose homeostatic mechanism, we incorporated the effects of insulin signalling in the insulin dependent tissues, for glucose uptake and intracellular glucose utilization. The rates of plasma glucose uptake and release by different tissues were obtained from Kim et al. (2007). A two compartment model of plasma glucose concentration and tissue glucose concentration was formulated. The plasma glucose compartment included the liver glucose release and uptake, glucose uptake by GLUT4 dependant tissues (ensemble of muscle, adipose and heart tissues), the constant glucose uptake by the non insulin dependent tissues (ensemble of brain, erythrocytes, GI track and other tissues), renal glucose extraction and the postprandial glucose appearance in the blood. The tissue glucose compartment included the GLUT4 dependant glucose uptake and the AKT-GSK3 dependant intracellular glucose utilization (Sumner, 2010). Since we were interested in just quantifying the effect of insulin signalling in the GLUT4 dependant tissue, only muscle, adipose and heart tissues were considered in tissue glucose compartment. The model for Glucose kinetic module are represented by Eq.(1) to (7).

GLUT4 mediated effect on glucose uptake was modeled based on the literature data (Nyman et al., 2011; Kim et al., 2007). It is also the function of the difference between the intracellular and extracellular glucose. It is represented as,

$$Glut4_{eff} = Vmx1\left(\frac{GLUT4^{n1}}{GLUT4^{n1}+km1^{n1}}\right)\left(\frac{Gp}{Gp+km2}-\frac{Gt}{Gt+km2}\right) \quad (1)$$

where, *Vmx1=7.5;* $K_{m1}$*=15mmol;* $n_1$*=0.8;* $k_{m2}$*=5 mmol; Gp*-Plasma Glucose concentration *(mmol), Gt*=Tissue glucose concentration *(mmol).* Insulin dependent intracellular glucose utilization was modeled as the function of AKT and GSK3 based on the ratio of glucose flux towards glycogen synthesis and glycolysis and the intracellular homeostasis with respect to extracellular glucose concentration. It is given as follows,

$$GSK3_{eff} = Vmx2 * \left(\frac{GSK3p^{n2}}{GSK3p^{n2}+km2^{n2}}\right) \quad (2)$$

$$AKT_{eff} = Vmx3 * \left(\frac{AKTp^{n3}}{AKTp^{n3}+km3^{n3}}\right) \quad (3)$$

$$ID_{gu} = \left(Vmx4 * GSK3_{eff} * \frac{Gt}{Kmx+Gt}\right) + \left(Vmx5 * AKT_{eff} * \frac{Gt}{Kmx+Gt}\right) \quad (4)$$

where, *Vmx2=10.5;* $k_{m2}$*=15,* $n_2$*=2, Vmx3=4;* $k_{m3}$*=2,* $n_3$*=2.* $K_{mx}$*=2.5 mmol; Vmx4=0.5 mmol/min; Vmx5=0.1mmol/min;* Eq.(2, 3 & 4) represents GSK3 effect, AKT effect and insulin dependent glucose utilization in the tissues, respectively. The function of liver was modelled in terms of a constant endogenous glucose release rate and a function representing the liver glucose uptake.The equation is as given below

$$Insu_{eff_{Liver}} = Vmx6\left(\frac{INSLIV^{n4}}{INSLIV^{n4}+km4^{n4}}\right)\left(\frac{Gp}{Gp+km5}\right) \quad (5)$$

where *Vmx6=13.5; km4=65 pmol/kg; n4=2; km5=12 mmol* and *INSLIV* is insulin concentration in liver. The rate of change in concentration of plasma glucose and equilibrating tissue's glucose concentration were represented by the following ODEs

$$\frac{Gp}{dt} * V_{bld} = EGR - Insu_{eff_{Liver}} - V1 * Glut4_{eff} - V2 * f(Gp) + Ra_{Glu} \quad (6)$$

$$\frac{Gt}{dt} * V_{tis} = \left(V1 * Glut4_{eff} - ID_{gu}\right) \quad (7)$$

where *EGR=1.125 mmol/min,* endogenous glucose release from liver and kidney*; V1=0.82 mmol/min,* maximum rate of glucose uptake by GLUT4 dependant tissues*; V2=0.5 mmol/min, f(Gp)* is the hill function of plasma glucose with *km=2 and n=4;* insulin independent glucose uptake*; Ra_Glu* is rate of meal glucose appearance in plasma, $V_{bld}$*=5.5 l,* Volume of blood*;* $V_{tis}$ is the volume of GLUT4 dependent tisuues. The parameters and the equations for renal glucose extraction and meal glucose appearance in plasma were adopted from the meal simulation model developed by Dalla Man, (2007).

### *3.3. Signalling Kinetic Module*

We have adopted the model for the insulin signalling pathway developed by Sedaghat et al., (2002). Authors have developed an integrated model by including subsystem models such as insulin receptor binding kinetics, receptor recycling and GLUT4 translocation. The model is also studied to demonstrated the effect of component concentration and parameters of insulin pathway on GLUT4 translocation (Giri et al., 2004). We have further extend this model to integrate the GLUT4 response to the cellular glucose uptake. The dynamic model of mTOR signalling was adopted from Vinod et al., (2009). The model quantified the effect of amino acids on the integrated mTOR-insulin signalling pathway, with and without the feedback mechanisms at various amino acid levels

(Kim, 2009; Lansard, et al., 2010). We further modified the model to incorporate the detailed model of insulin signalling pathway developed by Sedaghat et al., (2002), along with the cross talk and feedbacks and integrated it with the mTOR module from the model developed by Vinod et al., (2009). The integrated signalling model comprise of 35 ODEs, 35 rate equations and 80 parameters. The input of plasma insulin concentration was given as an input to the signalling module and the output concentrations of GLUT4, phosphorylated AKT and GSK3 were given as the input to the Glucose kinetics module.

## 4. PERTURBATION ANALYSIS

We have validated the integrated model with the normal physiological responses of plasma glucose levels under fasting and postprandial states. Each of the modules were individually validated to match the observed physiological responses in insulin secretion, plasma glucose levels, tissue glucose uptake and the signalling pathway with the data reported in literature (Dalla Man, 2007; Liu et al., 2008; Chew et al., 2009; Nyman et al., 2011; Kim et al., 2007).

The integrated Insulin-mTOR signalling model was subjected to parameter perturbation to quantify the effect of individual parameters on blood glucose levels. The rates of various reactions were perturbed on log-scale to access their contribution towards the regulation of plasma glucose homeostasis. Overall 75 rate parameters in the integrated network were perturbed by introducing the fold changes ranging from -100 to 100 folds with 15 intermediate step changes for each parameter (the negative fold change indicates fold reduction). The steady state plasma glucose concentration for a particular perturbation was recorded as the final response to a perturbation. The resulting change in glucose levels and corresponding fold change in parameter were used to rank the parameter effectiveness. We further characterized the glucose response for a perturbation in parameter in terms of pre-diabetic and diabetic responses. For the characterization of pre-diabetic and diabetic glucose levels, we followed the recommendations by the WHO (2006), such as 1) Pre-diabetic levels, also termed as Impaired Fasting Glucose (IFG) levels- Plasma glucose ranging from 6 mmol/l to 6.9 mmol/l (some reports also suggest glucose range of 5.6 mmol/l to 7 mmol/l). 2) Diabetic levels – Plasma glucose greater than 6.9 mmol/l, (i.e 7 mmol/l and above). The plasma glucose range from 4 mmol/l to 6 mmol/l was considered to be normal in this study. The plasma glucose levels below 4 mmol/l were considered to be hypoglycemic. We ranked the effectiveness of the parameters on the basis of the least fold change (it could be lower or higher than the normal parameter value) required to bring about pre-diabetic and diabetic response in the plasma glucose levels.

## 5. RESULTS

The results for the characterization of pre-diabetic and diabetic response and the corresponding fold changes required to elicit such a response are tabulated in Table 1. In the table, PD and D refer to fold changes leading to pre-diabetes (IFG) and diabetes, respectively. The ranking was done on the basis of the minimum fold change required in the parameter to elicit the diabetic response. The fold change on both the side of the abscissa (either reducing or increasing the parameter strength) was given equal significance. Of all the parameters tested for the fold change ranging from -100 to 100 folds, while 22 parameters demonstrated diabetic response, 31 parameters elicited pre-diabetic response for certain fold change in the respective parameter values.

Table-1)-Characterization of Pre-diabetic (PD) and Diabetic (D) fold changes in rate parameters and concentrations in integrated network.

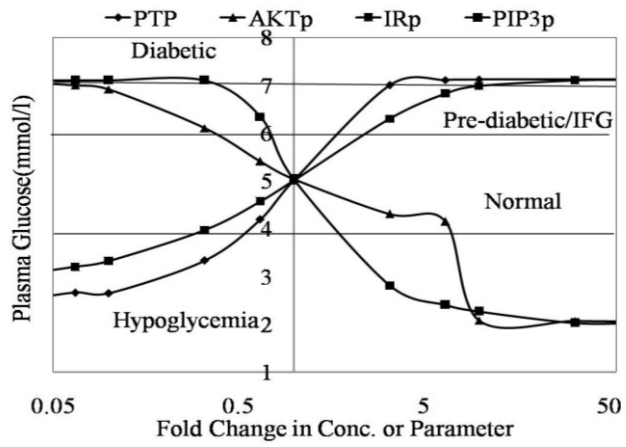
| Sr | Rank | Description | PD | D |
|---|---|---|---|---|
| 1 | 1 | PTP concentration | 2.16 | 3.21 |
| 2 | 2 | Phosphorylation of PIP2 to PIP3 | -1.38 | -3.84 |
| 3 | 3 | Conversion of RhebGTP to RhebGDP | -1.98 | -4.32 |
| 4 | 4 | Amino acid Influence on Rheb-GTP | 2.37 | 5.82 |
| 5 | 5 | Concentration of PTEN | 3.58 | 5.90 |
| 6 | 5 | Dephos. of PIP3 to PIP2 by PTEN | 3.58 | 5.90 |
| 7 | 6 | Tyrosine phosphorylation of IRS1 | -2.69 | -8.75 |
| 8 | 7 | Insulin Binding to Receptor | -2.72 | -8.94 |
| 9 | 8 | Unphosphorylation of IRS1 by PTP | 2.97 | 9.18 |
| 10 | 8 | Conc. of Phosphorylated Insulin receptor | 2.97 | 9.18 |
| 11 | 9 | Unphos. of surface receptor by IRS1 | 3.00 | 9.39 |
| 12 | 10 | Phosphorylation of AKT | -3.08 | -9.91 |
| 13 | 11 | Unphosphorylation of AKT | 3.17 | 9.95 |
| 14 | 12 | GSk3 phosphorylation | -3.33 | -24.2 |
| 15 | 13 | Glut4 degradation | 3.85 | 29.32 |
| 16 | 14 | GSk3 dephosphorylation | 3.49 | 30.29 |
| 17 | 15 | Dissociation of RhebGTP-FKBP38 | 17.38 | 32.15 |
| 18 | 16 | Unphosphorylation of PKC | -19.4 | -60.2 |
| 19 | 17 | Phosphorylation of PKC | 17.84 | 61.27 |
| 20 | 18 | Phosphorylation of S6k1 by mTOR-Raptor | 8.93 | 74.27 |
| 21 | 19 | Phosphorylation of PRAS40 | 9.08 | 84.07 |
| 22 | 20 | Unbinding of Intracellular receptors | 21.66 | 85.54 |
| 23 | 21 | Concentration of TSC | -6.83 | - |
| 24 | 22 | Degradation of S6K1p by PP2A | -9.91 | - |
| 25 | 23 | GLUT4 Expression | -9.92 | - |
| 26 | 24 | mTOR-raptor-PRAS40 complex | -16.9 | - |
| 27 | 25 | Unphosphorylation of PRAS40 | -21.2 | - |
| 28 | 26 | Phosphorylation of PIP2 to PIP3 | -32.7 | - |
| 29 | 27 | Concentration of SHIP | 36.77 | - |
| 30 | 28 | Inactivation opf PIP3 by SHIP | 36.77 | - |
| 31 | 29 | mTOR-raptor activation by Amino acids | 38.31 | - |

In the Insulin signalling pathway, the results of our analysis demonstrated that the PTP and PIP3 concentrations were most effective in terms of regulating the blood glucose uptake. Either by increasing the concentration of PTP by 3.0 folds or by increasing its inhibitory effect on IRS by 9 folds led to diabetic response. The parameters responsible for phosphorylation and dephosphorylation cycle of PIP3 also demonstrated higher effectiveness in regulating blood glucose levels. Reduction in phosphorylation of PIP2 to PIP3 by 4 fold demonstrated the diabetic effect. PTEN concentration also modulate the phosphorylation of PIP3 by deposphorylating it to PIP2, hence increase in its concentration by 6 folds elicit diabetic state. Besides this phosphorylation state of IRS1 and Insulin receptor also showed effective response, with 8 to 9 fold decrease in its rate of phosphorylation leading to diabetic state. It was also noted

that, by decreasing in phosphorylation of AKT or increasing its dephosphorylation by 10 folds shifted the signal response towards the diabetic state. Similarly, by decreasing the phosphorylation of GSK3 by 30 folds showed diabetic response. Whereas, around 60 fold change in PKC phosphorylation was required to elicit diabetic state.

Apart from this, the analysis of mTOR pathway demonstrated that by decreasing conversion of RhebGTP to RhebGDP and increasing amino acid influence on RhebGTP localization to the mTOR inhibitor complex by 4 to 5 folds independently, was most effective in leading to diabetic state. Moreover drastic increase in the dissociation of RhebGTP-FKBP38 complex, phosphorylation of S6K1p by mTOR-Raptor complex and unphosphorylation of PRAS40 also led to diabetes. Conclusively, in both the signalling pathways, it was noted that, only 2-3 fold change in above mentioned effective parameters led the system into pre-diabetic state or Impaired Fasting Glucose condition.Different kinds of plasma glucose responses for different fold changes in some of the effective parameters in insulin signalling and mTOR pathway are shown in Figure 3.

A.




B.

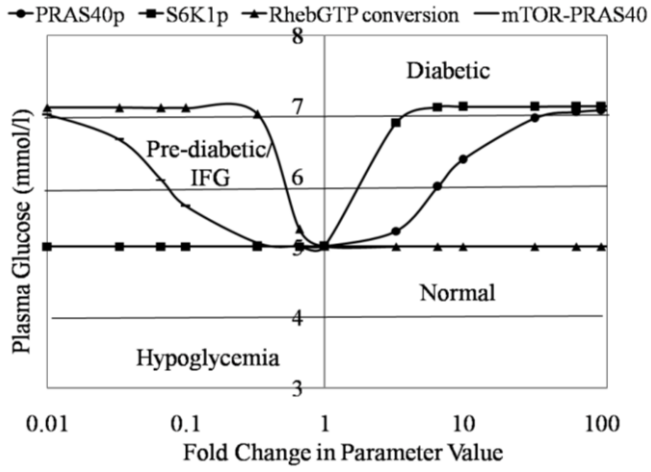



Figure 3. The steady state response of plasma glucose levels with respect to fold changes in key parameters of the signalling network. (A) Plasma Glucose response for effective parameters in Insulin signalling pathway. (B) Plasma Glucose response for effective parameters in mTOR signalling pathway.

## 6. DISCUSSION

Conventionally, glucose-insulin control system is most commonly used to model the glycemic control mechanism. Several mathematical models have been developed to understand glucose-insulin regulatory system (Athena et al., 2006). The most noticeable models are the minimal model containing minimal number of parameters which are widely used in physiological research to estimate glucose effectiveness and insulin sensitivity from intravenous glucose tolerance test (IVGTT) and oral glucose tolerance test (OGTT) data. Moreover, various models have been developed for whole body energy and metabolic homeostasis which illustrate the metabolism of fat, carbohydrates and amino acids based on the regulations by key regulatory hormones such as insulin and glucagon (Kim et al. and Ratchada et al. 2008). The models for mTOR signalling and effect of growth factors and amino acids on the pathway, also have been reported for yeast and mammalian cells (Wang and Krueger, 2010; Avruch et al., 2009; Vinod, 2009; Caron et al., 2010; Lansard, et al., 2010).

However, these models are only useful as far as the validation of certain physiological process are concerned and to quantify the gross effects of the hormonal regulations on metabolism. It is only in last decade that the detail molecular mechanisms are being deciphered underlying the hormonal regulation. It is recently that integrated models of insulin signalling and glucose metabolism are being developed (Liu et al., 2008; Chew et al., 2009; Nyman, 2011). The authors have integrated the insulin signalling to the blood glucose uptake with only GLUT4 as the integrating node for signalling and glucose uptake from blood. However, to study the physiological response in terms of disease states, a much detailed model which can account for the signalling regulation on glucose uptake, utilization, storage and disposal simultaneously is important. Through our integrated system of model, we have accounted for such details and analyzed the model response in terms of diabetic state with respect to perturbation in parameters. To our knowledge, this is first study of its kind in the literature, wherein the integrated insulin-mTOR signalling network is used to characterize pre-diabetic and diabetic states.

In insulin signalling pathway, the concentration and phosphorylation states of PTP and PIP3 appeared to be most effective in regulating blood glucose levels. Due its inhibitory effect at multiple sites i.e. serine phosphorylation of IRS1, receptor recycling and receptor phosphorylation of insulin receptors, PTP exerts greater control on the response of the pathway. The pathway has a feedback regulation on PTP by the AKT leading to positive feedback of AKT on the signalling pathway wherein activated AKT attenuate PTP action. This shows that if the positive feedback is decreased or lost the system becomes more prone towards diabetes due to enhanced PTP action. Similarly decreasing the PIP3 concentration due to unphosphorylation of PIP2 to PIP3 or PTEN action, also exhibited effective response. Since PIP3 is responsible for the activation of AKT and PKC, which further activates GLUT4 translocation and Glycogen synthesis, decreased PIP3 can affect both glucose uptake and utilization.

Moreover, decreasing the phosphorylation of AKT due to unphosphorylation of PIP3 reduces the positive feedback of AKT leading to enhanced action of PTP on the pathway, which further induces a sort of negative feedback in the upstream of the pathway leading to diabetic state. In the mTOR pathway, increasing RhebGTP concentration and its localization to the mTOR-raptor-FKBP38 complex, which is mediated by amino acids, enhances the activation of mTOR and S6K1p which is further known to inhibit IRS1 leading to attenuation of insulin signalling pathway (Vinod et al., 2009). Since there is no enough data for such individual rate perturbations in vivo, the effectiveness of some of these parameters mentioned above need to be validated in physiological experiments for further implications of these results.

## 7. CONCLUSIONS

The overall analysis shows that the signalling network operates on the brink of homeostatic and pre-diabetic state. Any smaller fold changes due to certain deregulation in these key parameters can disturb the homeostatic mechanisms. This implies the importance of the proper lifestyle and balanced nutrition. Any kind of chronic burden of the excess nutrient handling on the cellular systems may reduce the regulatory strength of the signalling network to maintain homeostasis. Several cohort studies have shown the adverse effect of high dose of amino acids and fat in the diet. In the above analysis it is observed that by increasing the strength of the parameter responsible for amino acid influence causes deregulation of glucose uptake by modulating insulin signalling pathway.

Furthermore, insulin signalling models can be integrated with the models of metabolic homeostasis which will depict dynamics of various regulatory mechanisms elicited by Insulin. Such alliance will help in elucidating how the metabolism is altered with changes in system parameters at signalling level. Since Insulin acts as the major hub in the metabolic network, the functioning of insulin is dependent on many of the network parameters which in turn are governed by respective signalling and genetic networks. Modelling these fundamental interactions is the prime task in system biology of Insulin Resistance which will help in analyzing various scenarios that lead to metabolic disorders. This approach has greater potential in identifying efficient drug targets that can help in controlling and curing diabetes and the morbidity associated with it.

## REFERENCES

A.H. Khan, J. E. Pessin (2002), Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways-*Diabetologia* ,45:1475–1483

Ahmad R. Sedaghat et al., (2002), A mathematical model of metabolic insulin signalling pathways- *Am J Physiol Endocrinol Metab* 283: E1084–E1101.

Alan R. Saltiel & C. Ronald Kahn (2001 )-Insulin signalling and the regulation of glucose and lipid metabolism-*Nature*, 414: (13) .

Ananda Basu and Chiara Dalla Man (2009)-Effects of Type 2 Diabetes on Insulin Secretion, Insulin Action, Glucose Effectiveness, and Postprandial Glucose Metabolism-*Diabetes Care*, 32( 5).

Athena Makroglou et al., (2006)-Mathematical models and software tools for the glucose insulin regulatory system and diabetes -*Applied Num Mathematics*, 56:559–573.

Chiara Dalla Man, Robert A. Rizza (2007)-Meal Simulation Model of the Glucose-Insulin System- *IEEE Transaction on Biomedical Engineering*, 54:10.

Claudio De Virgilio, Robbie Loewith, (2006), The TOR signalling network from yeast to man, *Int. Jour of Biochem & Cell Bio*.38 1476–1481

Etienne Caron et.al, (2010) A comprehensive map of the mTOR signalling network, *Mol Systems Biology* 6;.

Eunjung Kim (2009), Mechanisms of amino acid sensing in mTOR signalling pathway, *Nutrition Research and Practice* , 3(1), 64-71.

Giorgio Sesti (2006)- Pathophysiology of insulin resistance-*Best Practice & Res Clinl End & Met*, 20: 4, 665-679.

Guanyu Wang and Gerhard F. Krueger, (2010), Computational Analysis of mTOR Signalling Pathway: Bifurcation, Carcinogenesis, and Drug Discovery, *Anticancer Research* 30: 2683-2688.

Hiroaki Kitano et al., (2004), Metabolic Syndrome and Robustness Tradeoffs-*Diabetes*, 53.

Jayon Kim et al. (2007)-Multi-Scale Computational Model of Fuel Homeostasis During Exercise: Effect of Hormonal Control-*Annals of Biomedical Engineering*, 35 : 1.

Joseph Avruch, et al.,(2009), Amino acid regulation of TOR complex 1, *Am J Physiol Endocrinol Metab*, 296.

Keith N Frayn, (2010), *Metabolic Regulation-Human Perspective*-Book by Wiely-Blackwell publication UK.

Lopamudra Giri et al., (2004)- A steady state analysis indicates that negative feedback regulation of PTP1B by Akt elicits bistability in insulin-stimulated GLUT4 translocation- *Theor Biol and Med Modelling*, 1:2,1-16.

Marine Lansard, et al., (2010), Integration of insulin and amino acid signals that regulate hepatic metabolism-related gene expression in rainbow trout: role of TOR, *Amino Acids* 39:801–810

P.K. Vinod and K V Venkatesh (2009) - Quantification of the effect of amino acids on an integrated mTOR and insulin signalling pathway- *Mol. BioSyst* 5, 1163–1173.

Ratchada Pattaranit et al., (2008) Mathematical models of energy homeostasis- *J. R. Soc. Interface*,5:1119-1135.

Thomas Sumner-(2010) Sensitivity analysis in Systems biology modeling and its application to a Multi-scale model of blood Glucose homeostasis. *Thesis*

*WHO Report* (2006)- Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia.

W. Liu, F. Tang (2008), Modeling a simplified regulatory system of blood glucose at molecular levels, *J. Theor. Biol*. 252 608.

Weijiu Liu, Ching Chun Hsin, Fusheng Tang (2009)- A molecular mathematical model of glucose mobilization and uptake- *Mathematical Biosciences* 221: 121–129

Yin Hoon Chew, Yoke Lin Shia, Chew Tin Lee, (2009), Modeling of glucose regulation and insulin-signalling pathways- *Mol and Cellular Endocrinology* 303: 13–24.

Ying Leng, Hakan K.R. Karlsson and Juleen R. Zierath (2004)- Insulin Signalling Defects in Type 2 Diabetes, *Rev in Endo & Metabolic Disorder*; 5:111–117.