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Insulin signaling – mathematical modeling comes of age

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Signaling pathways that only a few years ago appeared simple and understandable, albeit far from complete, have evolved into very complex multi-layered networks of cellular control mechanisms, which in turn are integrated in a similarly complex whole-body level of control mechanisms. This complexity sets limits for classical biochemical reasoning, such that a correct and complete analysis of experimental data while taking the full complexity into account is not possible. In this Opinion we propose that mathematical modeling can be used as a tool in insulin signaling research, and we demonstrate how recent developments in modeling – and the integration of modeling in the experimental process – provide new possibilities to approach and decipher complex biological systems more efficiently.

Complex biological control systems and the need for mathematical modeling

Biological control systems are highly complex, and this complexity becomes more and more apparent as new layers of control are discovered. Insulin signaling, similarly to other signaling systems, was originally viewed as a linear cascade, but is now instead understood as a complex web of cross-interacting intermediates, sub-modules, and positive and negative feedback loops. To decipher such complex systems fully and correctly, new approaches, concepts and tools are required. Computational analysis using mathematical modeling has the potential to provide such tools.

Mathematical modeling can be used for different ends. One traditionally common approach is descriptive modeling, in which as many details as possible or necessary are included in order that the model is realistic and provides biologically meaningful predictions. Descriptive models allow one to compile available knowledge and to find internally coherent and realistic explanations for the data. A limitation with detailed descriptive models is associated with the fact that the mathematical description of a quantitative or dynamic model requires knowledge of concentrations and kinetic rate constants (see Glossary and Box 1). The values of such parameters are usually not known and, in combination with limited experimental data, predictions from detailed descriptive models therefore typically suffer from an unknown or arbitrarily high uncertainty. It is therefore of great interest to signaling network research that new tools and approaches to modeling have recently become available. These new techniques allow for stronger conclusions in the form of model rejections and more well-defined core predictions (Box 2).

In this Opinion, we draw attention to these developments with special regard to insulin signaling (Figure 1). We discuss modeling of the insulin control system, and highlight how recent modeling approaches have provided mechanistic insights at three different levels of the system: (i) insulin binding to its receptor, (ii) insulin signaling to cellular responses, and (iii) integration of intracellular insulin signaling with whole-body glucose homeostasis.

Glossary

AIC: Akaike information criterion. AIC is a tool for model selection which ranks models and takes model complexity into account. AIC does not provide statistical significance; not-picked models should therefore not be considered as rejected.
Bayesian modeling frameworks: modeling frameworks where dependencies in network structures are calculated using probabilities.
Boolean modeling frameworks: modeling frameworks where logic operators are used to simulate the model behavior. Fuzzy Boolean modeling frameworks extend the Boolean two-value logic (true or false) to include more possible values.
Conclusive modeling: data analysis using mathematical models with an ability to draw final conclusions such as rejections and core predictions.
Core prediction: a property that always has to be fulfilled given a specific model structure and available data. Core predictions are thus unique predictions and can be used to make decisions about new experiments.
EGF: epidermal growth factor signals through the MAP-kinase (Grb2–Sos–Ras–Raf–Mek–ERK) pathway also utilized by insulin (Figure 1).
GLUT4: insulin-regulated glucose transporter that is present in fat and muscle cells.
Identifiability: a model property. A model is practically identifiable if there are enough high-quality data to determine all model parameters with small uncertainty. Practical identifiability, unlike structural identifiability, takes the actual data into account.
IR: insulin receptor (Figure 1).
IRS1: the insulin receptor substrate-1, which is directly downstream the insulin receptor, is phosphorylated on tyrosine residues by the receptor, and then functions as a docking protein to transmit the insulin signal further (Figure 1).
Kinetic rate constant: determines the rate of a chemical reaction.
MAP kinase pathway: Canonical signaling pathway (Growth factor receptor–Grb2–Sos–Ras–Raf–Mek–ERK) for growth factor (including insulin) control of transcription via activation of ERK and phosphorylation of transcription factors.
Model structure: a set of equations that in a formalized way describe a mechanistic hypothesis.
mTOR: the mammalian target of rapamycin mediates insulin control of protein synthesis, mitochondrial function and autophagy according to the availability of amino acids and energy (Figure 1).
PKPD models: pharmacodynamic/pharmacokinetic models describe effects of drugs (pharmacodynamics) as well as mechanisms of distribution of drugs (pharmacokinetics).
PI3K: phosphatidylinositol 3-kinase is activated by binding to tyrosine - phosphorylated IRS1 and generates the second messenger phosphatidylinositol (3,4,5)-trisphosphate (Figure 1).
PKB: protein kinase B integrates insulin signaling to control of glucose uptake and other metabolic processes with growth control through activation of mTOR (Figure 1).
Scatchard plot: bound ligand/free ligand plotted against bound ligand. The plot is used to analyze the binding interactions between a ligand and its receptor.

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Box 1. Mathematical modeling of signaling systems using ordinary differential equations

Mathematical modeling of cellular signaling systems can be used as a formalized way of testing mechanistic hypotheses using experimental data and prior knowledge [48]. A mechanistic hypothesis corresponds to an idea of the mechanisms that are essential to produce the observed behavior in the experimental data. A single hypothesis typically contains many mathematical models that describe the same mechanism in slightly different ways, and a hypothesis cannot be rejected until all reasonable models within the hypothesis are rejected. For the translation of a hypothesis to such specific mathematical models, ordinary differential equations (ODEs) are commonly used. The ODEs include (i) states that, for example, correspond to concentrations or amounts of substances, or to membrane potential; (ii) parameters that, for example, correspond to the kinetic rate constants; and (iii) output variables that correspond to the experimental observations.

To illustrate how to translate a hypothesis into ODEs we look at a simple example from the early events in insulin signaling. An interesting signaling phenomenon is a characteristic overshoot behavior for the autophosphorylation of the insulin receptor (IR) in response to insulin (Figure I). The overshoot can arise, for example, from the internalization of the receptor followed by dephosphorylation and recycling of the receptor to the cell membrane (Figure II) [17,18].

To establish if these mechanisms are sufficient and/or necessary components of the observed signaling behavior, model-based hypothesis testing is useful. An ODE model corresponding to these mechanisms with assumptions of mass-action kinetics is:

$$[\dot{IR}] = -k1 \cdot [IR] \cdot [insulin] + kr \cdot [IRi] \quad [I]$$

$$[\dot{IRp}] = k1 \cdot [IR] \cdot [insulin] - k2 \cdot [IRp] \quad [II]$$

$$[\dot{IRi}] = k2 \cdot [IRp] - kr \cdot [IRi] \quad [III]$$

These ODEs express the time derivative – the rate of change in the concentrations of IR, IRp (phosphorylated IR) and IRi (internalized IR), respectively, The assumption that the receptor is non-phosphorylated before insulin-stimulation gives the initial values: $[IR](0) = 10$, $[IRp](0) = 0$, and $[IRi](0) = 0$. The output of the model is assumed to be proportional to the experimentally measured extent of phosphorylated receptor, $y = ky \cdot [IRp]$. We now have a ODE model with three states ($[IR]$, $[IRp]$, $[IRi]$), four parameters: $k1$ (the rate constant for activation of IR), $k2$ (the rate constant of internalization of IR), kr (the rate constant for recycling of IR to the plasma membrane) and ky (scaling parameter for measurements), one input signal ($[insulin]$), and one output signal (y). The model can be used to simulate early events of insulin signaling for different values of the parameters and the input signal.

The values of the parameters, in other words the kinetic rate constants, are difficult to determine experimentally. These values can, however, be estimated using the model, the experimental data, and realistic limits of the parameter values by testing different values within the limits, to evaluate the agreement between the simulated output of the model and the experimental data.

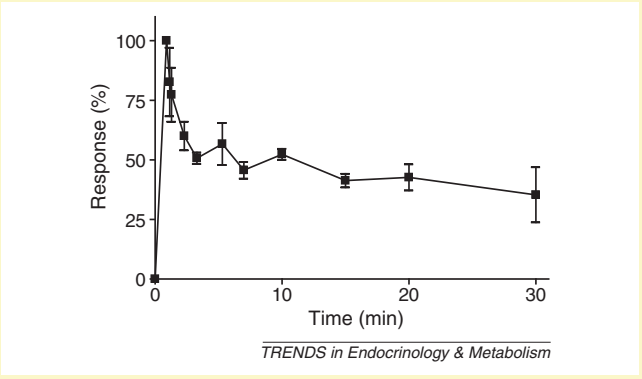


Figure I. The extent of phosphorylation of the insulin receptor in response to 100 nM of insulin shows a characteristic overshoot behavior.

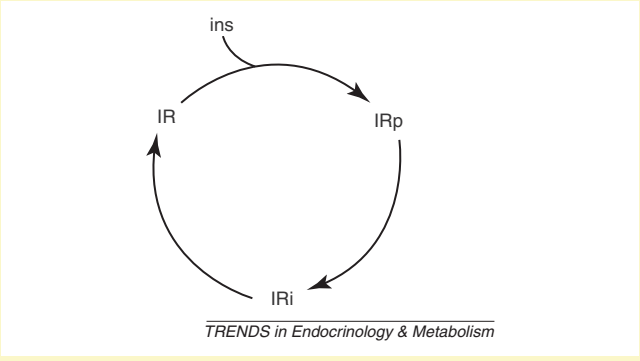


Figure II. A simple sketch of how the insulin receptor (IR) is phosphorylated (IRp) in response to insulin (ins), then internalized and dephosphorylated (IRi), and finally recycled back to the cell membrane.

Modeling insulin binding to the insulin receptor

Binding of insulin to, and dissociation from, the insulin receptor (IR) has been modeled for several decades [1–5]. State-of-the-art conclusions are based on experiments using labeled insulin, which display a curvilinear Scatchard plot for insulin binding and a bell-shaped dissociation curve (which implies a loss of accelerated dissociation of the radioactive insulin tracer at high concentrations of non-labeled insulin) (Figure 2a). Mathematical modeling by Kiselyov *et al.* [5] has recently provided a mechanistic and quantitative explanation for these complex data. In the proposed model (Figure 2b), two identical insulin-binding α subunits exhibit two binding sites each. Following insulin binding to one subunit, oscillations in the structure of the receptor allow for the same insulin molecule to bind also to the other subunit. This cross-linking of the two α subunits allows both the two remaining free binding sites to bind one insulin molecule each. The assumption that only cross-linked receptors can bind three insulin molecules enabled the model to exhibit the characteristic deceleration of insulin dissociation at high concentrations. The complete model involves 35 states of the receptor but, at physiological concentrations of insulin, when the probability that more than one molecule of insulin binds to the receptor is low, the model can be simplified to a simple four-state model [5].

The model by Kiselyov *et al.* [5] has been developed using a non-conclusive modeling approach (Box 2) but is nonetheless the first to provide a mechanistic and quantitative explanation for the available experimental data. The work was preceded by other efforts, and the concept of cross-linking had earlier been introduced in two slightly different forms without implementing the concept in mathematical models [6,7]. The concepts in [6,7] had also been implemented in modeling and fitted to insulin–IR binding data, but no quantitative comparison with the dissociation data was reported [4]. Another interesting model of insulin binding includes receptor aggregation [3]. The aggregation-based model exhibits both positive and negative cooperativity for insulin–IR binding, but the model does not explain the complex dissociation data above.

Box 2. Conclusive versus non-conclusive modeling

In a conclusive model-based approach, modeling and experimental work are integrated and strong conclusions can be drawn and reported. The important advance over more traditional non-conclusive modeling approaches, where a single model is presented and analyzed, is the ability to draw strong conclusions (Figure I). This ability relies on three pillars: (i) integration of mathematical modeling with experiments in an iterative fashion, (ii) formulation of successive model structures that can be rejected, and (iii) introduction of core predictions, which allow for model analysis that is independent of specific parameter values [18]. Regarding (i), the main difference is that conclusive modeling is primarily a form of data-analysis, as opposed to constructing models as an end in itself. Regarding (ii), traditional descriptive and grey-box modeling has typically centered on one, or at most a few, models that are developed to be as realistic as possible and then analyzed in depth. Whereas such modeling allows for understanding of the model, comparison and rejection of many different smaller models allow for insight into the underlying system – with respect to which interactions are necessary/sufficient/redundant to serve as a mechanistic explanation for the observed dynamics. Rejection of a model is a strong and final statement because rejections are not revised by more data (unless, of course, previous data were erroneous), whereas a model is only acceptable until evidence to the contrary is obtained [16]. Even though rejections are part of traditional non-conclusive modeling, such rejections may not be conclusive and are rarely reported. Regarding (iii), core predictions are predictions that are valid for all parameters and model combinations that agree with the experimental observations, whereas a traditional single-value parameter simulation is only valid for a model with specified parameter values [18]. This is an important difference because available experimental data on insulin signaling do not yet allow for a unique estimation of all parameter values – a problem referred to as unidentifiability. Importantly, predictions from unidentifiable models may be arbitrarily wrong or uncertain even though the model agrees with the validation data. Conversely, a core prediction is a strong and final statement regarding a property that must be fulfilled if the model structure should serve as an explanation of the data.

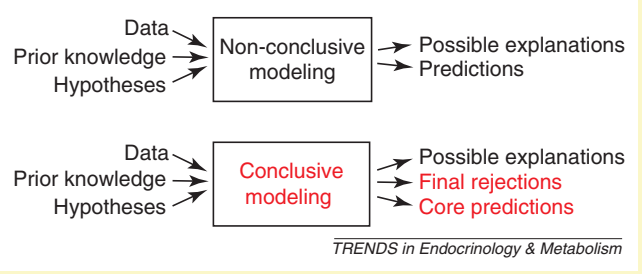


Figure I. Modeling provides a formalized method for setting up hypotheses from prior knowledge and experimental data. Non-conclusive modeling focuses on the creation of a model and on gathering all available information about a system in a single model. Conclusive modeling, on the other hand, focuses on how to use conclusions from modeling in the experimental work by using minimal models to draw final conclusions, such as model rejections and core predictions.

In summary, mathematical modeling has provided tools to gain mechanistic insight from the complex behavior observed for insulin binding to and dissociation from its receptor. These non-trivial mechanistic understandings could not have been obtained using biochemical reasoning alone: reasoning could not, for example, have proved that the rather complex model for insulin–IR interactions in [5] can explain the data in a quantitative fashion and that simpler models are insufficient as explanations.

Modeling IR internalization reveals a role in insulin signaling

Experimental data on insulin–IR binding and dissociation are often obtained at $<15\ ^{\circ}C$, to minimize endocytosis of the receptor. However, receptor internalization and recycling are important components of signal transduction by insulin and these processes have also been modeled. An early model with two pools of IR, one intracellular and one in the plasma membrane, fits well to experimental data on insulin effects in a BC3H1 muscle cell line [8]. The same data together with a slightly different hypothesis have been used to build a model that also includes receptor synthesis and degradation [9]. The model was further fitted to a dataset describing insulin binding to receptors (at $4\ ^{\circ}C$, when internalization was blocked) in a muscle cell line undergoing differentiation over a period of 14 days. However, cell transformations during differentiation affect insulin signaling, and the results in [9] are therefore hard to interpret. Effects of changing the amount of IR have been simulated using a complex model for internalization of IR [10]. Because patients with type 2 diabetes (T2D) and obesity exhibit a reduced number of receptors [11,12] the effect of such a reduction is an important aspect to understand. However, it was not clearly declared how the parameters of the complex internalization model were determined and the model [10] showed no agreement with any experimental data, and therefore the accuracy of the model structure cannot be evaluated.

The dynamics of autophosphorylation and internalization of IR have been studied experimentally in Fao hepatoma cells and using mathematical modeling; a simple model of internalization was fitted to a time-series of internalization data with good agreement [13]. Three more complex model structures were fitted to the same data to investigate the dynamics of the internalization process [14]. All the models agreed with the data, and the authors used the Akaike information criterion (AIC) [15] to judge between the models. AIC is designed to provide a quick choice between competing models and accounts for the number of datapoints, the differences between model simulation and datapoints, and the complexity of the model. However, AIC is not associated with a statistical significance and rejections based on AIC are thus not compatible with a conclusive modeling approach [16].

The importance of IR internalization for signal transduction was recently examined thoroughly using model-based data analysis [17,18]. The original observation was an overshoot of the autophosphorylation of IR and of the IR-catalyzed tyrosine phosphorylation of the downstream signal mediator insulin receptor substrate-1 (IRS1) [17,18]. The phosphorylation of both IR and IRS1 increased rapidly upon addition of insulin, with a peak value after about one minute, when the phosphorylation decreased again to an intermediate quasi-steady-state level (Figure 3). This overshoot behavior implies that a negative feedback is elicited, and four fundamentally different feedbacks were formulated that could explain the overshoot behavior – the hypotheses *Md, Mm, Mf, Mi* (Figure 3). Integration of model-based data analysis with experimental tests, in an iterative fashion, revealed that the feedback

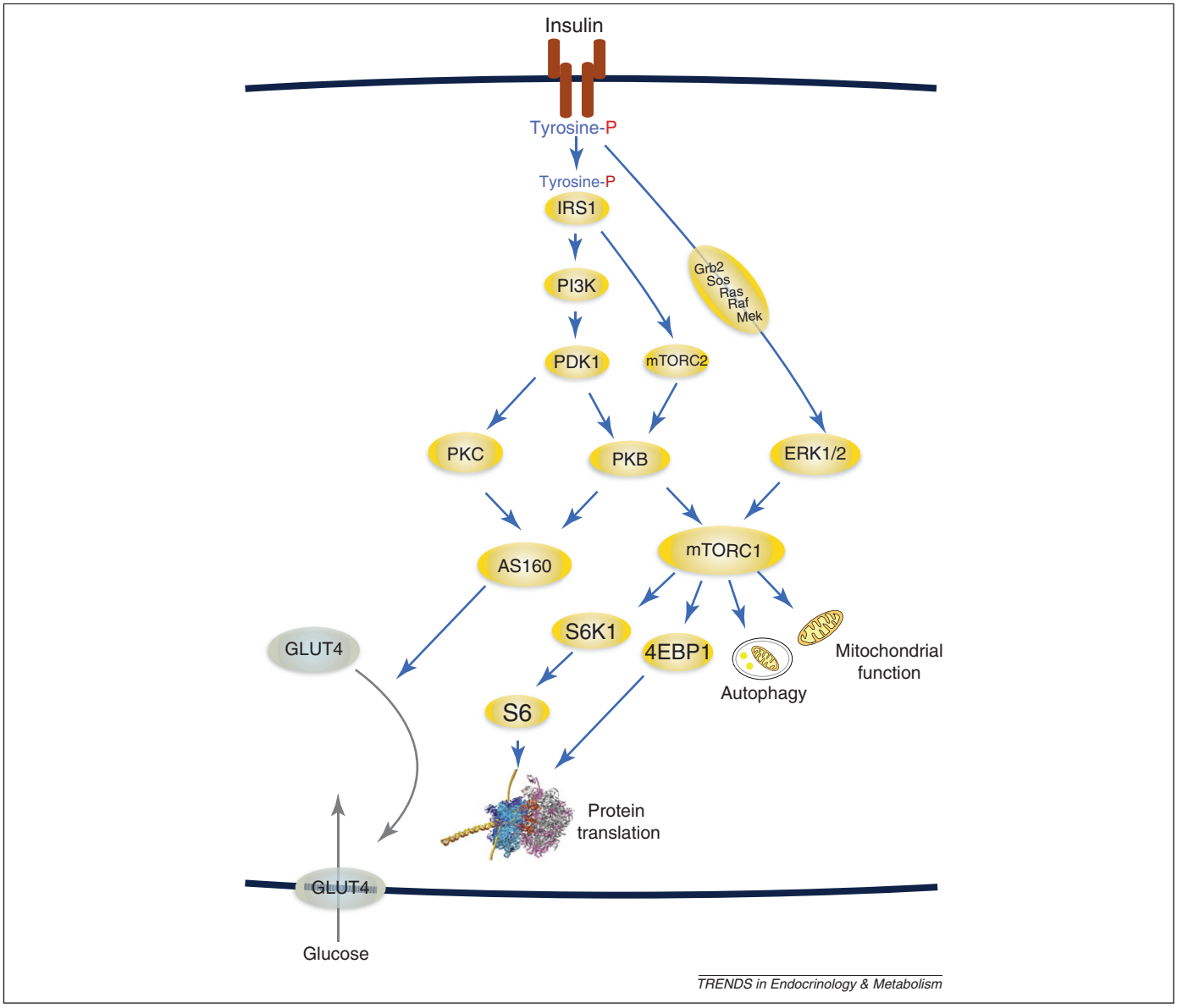


Figure 1. Schematic outline of insulin signaling pathways. Insulin binding to the insulin receptor (IR, brown) causes autophosphorylation of IR at tyrosine. Thus activated, IR will phosphorylate the insulin receptor substrate-1 (IRS1) at tyrosine to create binding sites for SH2 domain-containing proteins such as the phosphatidylinositol 3-kinase (PI3K) or growth factor receptor-bound protein-2 (Grb2). Activated PI3kinase will phosphorylate phosphoinositides in the cell membrane, allowing phosphoinositide-dependent kinase-1 (PDK1) to phosphorylate and activate protein kinase B (PKB) and protein kinase Cλ/ζ (PKC). Thus activated PKB can activate mammalian target of rapamycin (mTOR) in complex with raptor, through which insulin can control protein synthesis, autophagy and mitochondrial function. Through activation of Grb2 and Sos–Ras–Raf–Mek insulin also controls extracellular-related kinase-1/2 (ERK1/2), which in turn controls mTOR and different transcription factors. Blue arrows indicate downstream signaling by insulin, black arrow indicates translocation of insulin-regulated glucose transporter-4 (GLUT4) from an intracellular location to the plasma membrane, and P indicates phosphate.

cannot consist of insulin degradation (*Md*) because too little insulin was degraded (Figure 3). Neither can the feedback consist only of competitive inhibition at the plasma membrane (*Mm*), nor only of negative feedback from downstream signaling intermediates (*Mf*), because experimental blocking of internalization extinguished the overshoot behavior. This showed that internalization is a necessary component of the feedback. However, further iterations between conclusive modeling and experiments also concluded that internalization alone (*Mi*) cannot be the actual feedback because too little IR was internalized to fulfill the requirements of the model (Figure 3). Internalization is therefore a necessary but not sufficient part of the feedback [18]. This conundrum can be resolved if the feedback that gives rise to the overshoot behavior emanates from downstream signaling intermediates elicited by the internalized IR (Figure 3). Such a model explains all the available experimental data [18], but it should be emphasized that the final model is a suggestion and not a conclusion, and the model might be rejected when more data are collected. The above-described conclusive model-analysis approach goes beyond the traditional approach of creating a single model to describe the available experimental data (Box 2).

In summary, with regards to internalization of IR and its role in the early phase of insulin signaling, mathematical modeling has demonstrated that it has come of age as a potent research tool. The current understanding of IR

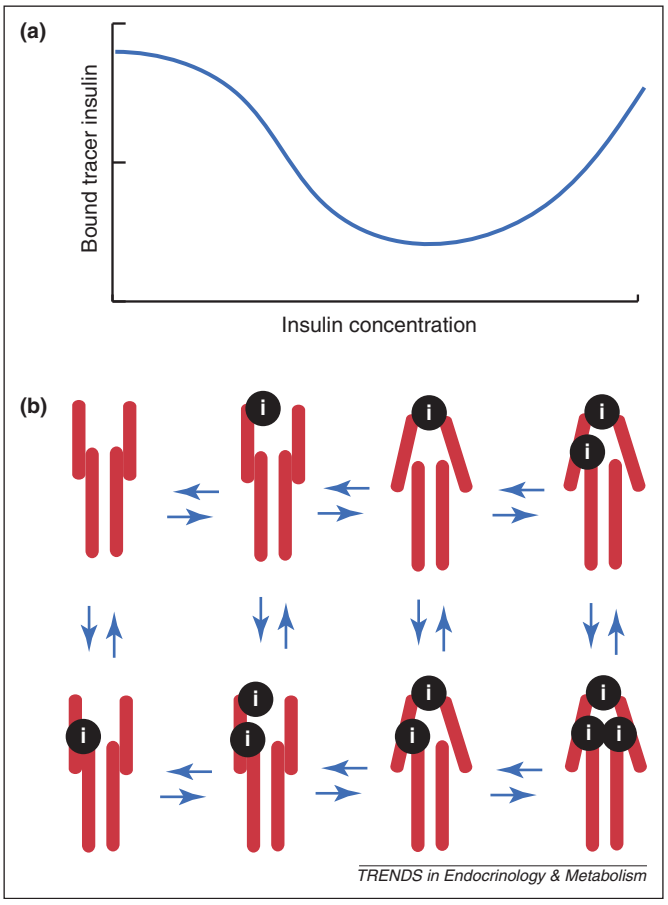


Figure 2. Insulin binding to IR. **(a)** Illustration of the bell-shaped insulin concentration-dependency of the insulin–IR dissociation rate [5]. **(b)** Schematic illustration of the main characteristics of the Kiselyov model [5] that describes the binding of insulin (black dot) to the α subunits of IR and the subsequent crosslinking of the α subunits. Increasing insulin concentration increases the number of insulin molecules that bind to each receptor. At physiological insulin concentrations only one insulin molecule binds to the receptor and the model can be simplified.

internalization – as a necessary but not by itself sufficient part of a feedback that kicks in within one minute – could not have been reached without modeling: necessity was implied by the experimental data, but model-based data analysis was required to reveal that the observed extent of internalization alone is not sufficient to provide the observed dynamics. Insulin signaling in adipocytes thus involves feedback mechanisms that depend on IR internalization to limit downstream signaling to, for example, glucose uptake. Indeed, it was experimentally found that blockade of the feedback caused a several-fold increase in signaling in response to insulin [18]. The identified feedback thus constitutes a novel mechanism that may be utilized to enhance insulin-sensitivity in insulin-resistant states.

Modeling downstream signaling and crosstalk

Insulin–IR binding and internalization initiate downstream signaling that has been analyzed using mathematical modeling. Based on previous models of insulin–IR binding [3,9] and regulation of the insulin sensitive glucose transporter (GLUT4) [19], Sedaghat *et al.* [20] built a model of insulin signaling. The model contains several of the most accepted mechanisms and intermediates in downstream signaling to control of glucose uptake, such as activation of phosphoinositide 3-kinase (PI3K), phosphorylation of protein kinase B (PKB), and translocation of GLUT4 to the plasma membrane. As the only comprehensive model of insulin signaling it has reigned for several years, even though it has problematic shortcomings. The model is based on limited amounts of experimental data obtained in different experimental systems and settings, with parameter values chosen somewhat arbitrarily, and only a single value for each parameter was examined. The benefits of a core-prediction approach with strong final conclusions and validity for a complete model structure therefore do not apply, and all model predictions are of the epistemological character of 'it can be this way but also in some other way'. Moreover, many of the parameter values are unrealistic, with for instance initial concentrations of 10^{-15} M – corresponding to less than one molecule per cell. Several of these problems are concealed because model predictions were only compared with data in scaled arbitrary units.

In a steady-state analysis of the insulin signaling network [21], many of the signaling intermediates and parameters were re-used from the Sedaghat model. The main finding was bi-stability, in other words, the notion that two steady-states might coexist for given parameters and constant insulin stimulation. However, no experimental validation of the results was presented. The Sedaghat model has also been extended to include amino acids, the mammalian target of rapamycin (mTOR), and some other aspects of the signaling network [22]. New parameters were 'suitably assumed' and the model was fitted to a limited amount of data compared to the complexity of the model. The lack of data is even more conspicuous in a model that presents insulin signaling mainly to illustrate a theoretical model reduction approach with no data or biological insights reported [23].

The perhaps most modeled of all signaling networks is epidermal growth factor (EGF) signaling and activation of the MAP kinase pathway [24]. The same MAP kinase pathway is also under insulin control (Figure 1). The EGF and insulin signaling networks are thus sub-networks in a larger network of overlapping, or crosstalking, sub-networks. One of the most intriguing problems to understand in signaling is that of crosstalk versus pathway specificity: how downstream targets distinguish between different inputs despite sharing intermediary signals. Modeling has examined specificity obtained by, for example, differences in the transient profiles (two ligands stimulate the same end targets in a transient or a sustained manner), by localization, and by sequestration (specificity through binding to different scaffolds) [25,26]. In a first larger model to include both EGF and insulin signaling [27], insulin-specific aspects were developed by selecting key signaling intermediates and using previously modeled interactions. The model [27] was fitted to new data from HEK cells and predictions were used to validate the model. However, conclusions from rejected models in the development of the presented model were not reported.

Models described so far are based on ordinary differential equations (ODEs) (Box 1), which describe dynamic behaviors and are thus preferred when used with time-resolved, informative data. Other modeling frameworks

Data exhibiting rapid overshoot
in phosphorylation of IR and IRS1

Identification of hypotheses that
can produce overshoot behavior

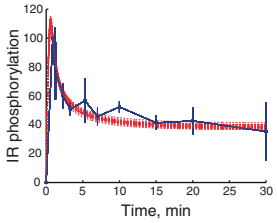
Md - insulin degradation

Mm - sequestration of IR
at the plasma membrane

Mf - feedback signal to
dephosphorylation of IR

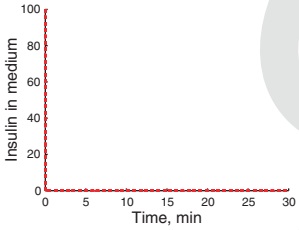
Mi - endocytosis of activated IR

Iteration 1



Md, Mm, Mf, Mi
accepted

Core prediction for Md:
simulations for all acceptable parameters:
>95% insulin degraded
within 1 min



Iteration 2

Data showing negligible insulin
degradation

Md rejected

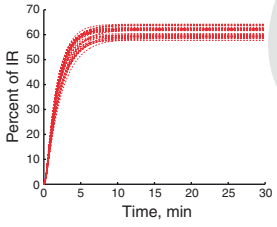
Properties that distinguishes the
remaining hypotheses:
only Mi involves endocytosis of IR

Iteration 3

Data showing that IR endocytosis
is required

Mm and Mf rejected

Core prediction for Mi:
simulations for all acceptable parameters:
between 55 and 65% of IR
internalized
at steady-state



Iteration 4

Data showing < 5% of IR internalized
at steady state

Mi rejected

i.e. all hypotheses rejected

New hypothesis:
Mif = (Mi + Mf) - endocytosis of activated IR
and generation of feedback
to IR dephosphorylation

Iteration 5

Mif passes all tests
Mif accepted

More experimental data and
continued refinement of hypothesis

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Figure 3. Example of conclusive modeling: IR internalization and the early phase of insulin signaling. A schematic illustration of the workings of conclusive modeling when integrated with experiments [18]. Initial experimental data demonstrated an overshoot in the phosphorylation of IR and IRS1 in response to insulin. Four different hypotheses for insulin signaling were formulated that could produce an overshoot behavior. Consecutive iterations between experiments and conclusive model analyses found that all four hypotheses had to be rejected. Internalization of IR is required, however, and the corresponding hypothesis Mi can produce an overshoot behavior, but the hypothesis requires that >55% of IR is internalized at steady state. However, it was found experimentally that <5% of IR is internalized, and the hypothesis was rejected. The inserted graphs show some simulations for extreme values of all acceptable parameters, in other words all the parameters that made the corresponding hypothesis acceptable up to that point. Because all acceptable parameters were examined, a uniquely identified prediction can be made that is independent of parameter values – which is referred to as a core prediction. By combining the requirement for internalization (hypothesis Mi) and the generation of a feedback signal to enhance the

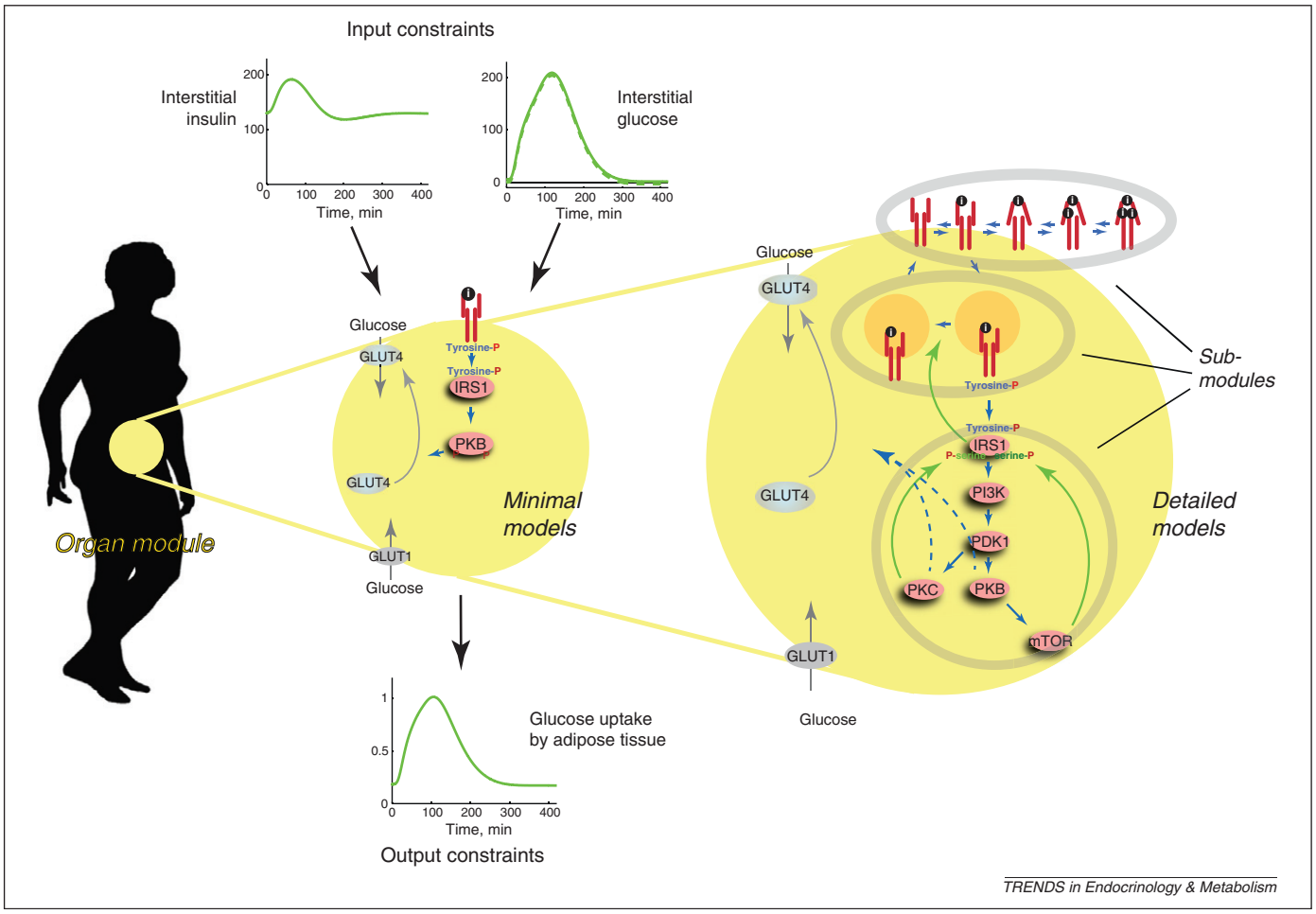

Figure 4. Integration of insulin signaling and glucose homeostasis using constraint-based multi-level modeling. In [41], minimal models for insulin signaling were analyzed using constraints from a whole-body glucose homeostasis model developed by Dalla Man *et al.* [39]. Constraints are properties that need to be fulfilled for the minimal model of insulin signaling (middle part) to fit with the whole-body level (left part). The input constraints are the interstitial glucose and insulin concentrations of the tissue and the output constraint is the glucose uptake by the minimal model (middle part). All constraints were obtained as simulated responses to a meal from the whole-body-level model (left part). The minimal model was further expanded with sub-modules (right part) with details of insulin signaling (top to bottom) from the Kiselyov model [5], the Brännmark model [18], and a more detailed model for downstream signaling that includes several feedbacks.

are preferable for more high-throughput and less informative data with no or poor time resolution. Such frameworks are typically based on logic (e.g. Boolean or fuzzy Boolean) or probabilistic (e.g. Bayesian) relations. A main benefit of Boolean modeling is that model structures are simple and kinetic parameters are absent (and therefore do not need to be estimated). A recent large-scale Boolean model uses a random update approach combined with many simulations [28]. The randomness mimics a situation where a single cell presents a unique response, but with a sufficiently large number of cells a reproducible continuous time-series can be obtained.

Modeling of links to the whole body

Two of the main reasons for studying insulin signaling are its central function in glucose (energy) homeostasis and the corresponding malfunctioning in T2D. These reasons both argue for the importance of linking models of insulin signaling to models of whole-body glucose homeostasis. Such multi-level models are important because diseases emanate from, and drugs act at, the intracellular molecular level, whereas diseases are manifested and diagnosed at the whole-body level. Such a combination can effectively be achieved by insertion of intracellular models as modules (i.e. components) in a multi-level whole-body model. Such hierarchical, module-based, modeling is common in engineering [29] and some preliminary attempts to link insulin signaling models with glucose homeostasis models have recently been published.

Two fairly comprehensive models [30,31] that link whole-body glucose homeostasis with intracellular dynamics include several hormones and metabolites, at the whole-body level, and the major organs and various metabolic subsystems, at the subcellular level. However, no mechanisms of insulin signaling are present in these models, and insulin directly affects metabolism using various phenomenological expressions. Models by the company Entelos [32] include a similarly detailed whole-body level and also some of the signaling intermediates of the insulin signaling pathway. These models are being used by drug

dephosphorylation of the internalized IR (hypothesis Mf), a model that explains all available experimental data is identified (hypothesis Mif). This conclusive modeling approach has rejected several plausible hypotheses and identified an acceptable hypothesis for the early phase of insulin signaling. The rejected models will remain rejected, but the accepted model may be rejected later, when more data become available.

companies in projects of pharmaceutical drug development, but are commercial and are not open to scientific scrutiny. A more qualitatively oriented multi-level model demonstrated that a propensity for developing the metabolic syndrome can be the result of sensitivity to long-term over-eating, which in turn is due to an evolutionarily developed robustness towards starvation and irregular food supply [33].

In contrast to models with simple or almost non-existent insulin signaling but detailed whole-body dynamics, there are also models with more detailed insulin signaling but a simple whole-body description. Koscherrek *et al.* [23] linked models of insulin binding and degradation, but no downstream signaling, with models for plasma concentration of insulin. Model parameters were from published data and the resulting model was compared with data from rat hepatocytes. Similarly, a multi-level model that includes signaling from the Sedaghat model [20] contains plasma insulin and glucagon concentrations, insulin and glucagon signaling, and glucose production and utilization [34,35]. Chew *et al.* [36] linked the Sedaghat model with a simple model for whole-body glucose homeostasis [37]. A general weakness of these models is that they rely on the Sedaghat model [20] with its arbitrarily guessed parameter values and other inherent problems discussed above. These studies consequently fail to draw strong conclusions in terms of rejections and unique predictions.

Simple whole-body models of glucose–insulin interplay have been available for several decades, in particular in the field of pharmacodynamical/pharmacokinetical modeling (PKPD). For instance, a simple minimal model from 1979 [38] is still frequently used. Different variations and extensions of such models have been developed, and an important recent example is by Dalla Man *et al.* [39]. Based on high-quality triple-tracer data from >200 healthy subjects, the major fluxes of glucose and insulin during a meal were estimated in a largely model-free way. A version of the model is accepted by the US Food and Drug Administration (FDA) as a replacement for animal testing when certifying particular insulin treatments in type 1 diabetes [40]. This acceptance is an important example of how mathematical modeling is coming of age as an important research tool. The Dalla Man model is nonetheless of limited use in, for example, drug screening or identification of drug targets in T2D because intracellular details regarding signaling and metabolism are lacking.

The Dalla Man model and the corresponding high-quality data allow for conclusive modeling when developing multi-level models that link insulin signaling with whole-body homeostasis. Here, the experimentally determined fluxes between organs act as constraints on the allowed input/output signals and enter with the same status as experimental data in the development of the insulin signaling model (Figure 4) [41]. This allows one to draw conclusions regarding the detailed subsystem, exactly as if one had modeled only that subsystem. These constraints also ensure that the developed model will act as a replaceable module in the whole-body model. Using such constraints and a conclusive multi-level modeling approach, a minimal model for insulin signaling in an adipose tissue module was developed [41]. Development of the model concluded that the insulin-regulated uptake of glucose by freshly isolated human adipocytes cannot simply be scaled up to match the observed adipose tissue input–output profile of glucose. A combination of stress-induced translocation of glucose transporter during cell isolation and insulin effects on the bloodflow was proposed as an alternative to direct scaling, and such a model could explain the available data at both the intracellular and the whole-body levels. A limitation with this approach is that the uncertainties of the input constraints from the Dalla Man model were not accounted for. Note that the conclusive modeling approach, in this setting, also does not prove that the final model is correct. The resulting multi-level model [41] was further extended to include all the three earlier mentioned state-of-the-art models: the detailed model of insulin binding to its receptor by Kiselyov *et al.* [5], the Brännmark model of receptor internalization/feedback signaling [18], and the Dalla Man model of whole-body glucose homeostasis [39] (Figure 4).

Concluding remarks

In this Opinion we have shown how recent progress in mathematical modeling has advanced understanding of insulin signaling and whole-body glucose homeostasis. The non-linear binding and dissociation characteristics of insulin–IR interactions have received a quantitative and non-trivial mechanistic explanation. Similarly, iterations between experiments and conclusive modeling have allowed for the conclusion that internalization of IR is a necessary but not by itself sufficient component of a strong and rapid feedback. At the same time, models of downstream signaling and crosstalk have acquired more complexity and realism. Whole-body models of glucose homeostasis are already used in drug certifications, and a recent multi-level model integrates state-of-the-art understanding of insulin–IR binding, IR internalization and signaling as well as reciprocal interactions with whole-body homeostasis.

Comparison with some state-of-the-art models for other biological systems can also be of value. Related multi-level models exist for the human heart, and these span the kinetics of ion channels to the spatiotemporal dynamics of the organ [42]. These models are generally more advanced. However, that area of research has not come as far in terms of conclusive modeling, and has not yet achieved a correspondence to the FDA acceptance of the Dalla Man model. Other success stories exist for yeast [43] and other simpler organisms. There, modeling has often moved beyond the state-of-the-art in models of insulin signaling and has included modeling of cell shape and associated biophysics [44], or stochastic, or even single-molecule dynamics in single cells [45–47].

The difficulties posed by the inherent complexity of biology require that the tools offered by mathematical modeling continue to advance so as to provide the basis for new treatments of diseases. Potent tools are already available, but major stumbling blocks are the separate cultures of experimentalists and mathematicians and the consequent lack of coherent data suitable for modeling. As research into the mechanisms of insulin action and the pathogenic mechanisms of T2D increasingly makes use of

mathematical modeling, and success stories accrue, we predict that mathematical modeling in the near future will move into the experimental laboratories and will become indispensable for experimental scientists.

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