Requirements for multi-level systems pharmacology models to reach end-usage: the case of type 2 diabetes

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We are currently in the middle of a major shift in biomedical research: unprecedented and rapidly growing amounts of data may be obtained today, from *in vitro*, *in vivo* and clinical studies, at molecular, physiological and clinical levels. To make use of these large-scale, multi-level datasets, corresponding multi-level mathematical models are needed, i.e. models that simultaneously capture multiple layers of the biological, physiological and disease-level organization (also referred to as quantitative systems pharmacology—QSP—models). However, today’s multi-level models are not yet embedded in end-usage applications, neither in drug research and development nor in the clinic. Given the expectations and claims made historically, this seemingly slow adoption may seem surprising. Therefore, we herein consider a specific example—type 2 diabetes—and critically review the current status and identify key remaining steps for these models to become mainstream in the future. This overview reveals how, today, we may use models to ask scientific questions concerning, e.g., the cellular origin of insulin resistance, and how this translates to the whole-body level and short-term meal responses. However, before these multi-level models can become truly useful, they need to be linked with the capabilities of other important existing models, in order to make them ‘personalized’ (e.g. specific to certain patient phenotypes) and capable of describing long-term disease progression. To be useful in drug development, it is also critical that the developed models and their underlying data and assumptions are easily accessible. For clinical end-usage, in addition, model links to decision-support systems combined with the engagement of other disciplines are needed to create user-friendly and cost-efficient software packages.

1. Introduction

Mathematical models have been widely used in biology and medicine for several decades, and have, in certain areas, already gained widespread acceptance. The first and perhaps still most well-known example is the model developed by Hodgkin and Huxley in 1952 [1]. This model is noteworthy both because it illustrated, in quantitative terms, how the membrane potential in living cells can be viewed as an ordinary electrical circuit, and because it also is the first mathematical model that formed the basis for a Nobel Prize in physiology (in 1963). Since the original Hodgkin and Huxley model, this research field centred on electrical activity in cells has considered mathematical modelling as a necessary component
of the research process. Examples of such continued research include topics such as calcium channel dynamics in neurons [2], for which Katz received a Nobel Prize in 1970, and research on the electrical activity of the heart [3]. Another example where models have shown to be of importance comes from the field of diabetes research, where a glucose homeostasis model has received regulatory acceptance by the US Food and Drug Administration as a viable replacement for test animals for certain insulin treatments, including closed-loop algorithms [4,5]—something that has led to a formidable explosion in the industry producing devices for insulin dosage.

An important aspect of the more recent progression in modelling in biology is that multi-level models, i.e. models that simultaneously capture several layers of biological organization, are being developed. Considering research on the heart, current multi-level models describe simultaneously the detailed functions of various ion channels at an intracellular level, the resulting membrane potential propagation across myocytes, and, in some cases, the further integration of these processes into the formation of an actual heartbeat across the entire myocardium [6]. This research has now reached such a level of maturity that the models are being used and accepted as arguments for safety certifications of new drugs [7,8]. Considering research in diabetes, multi-level models have helped us unravel how insulin resistance, i.e. a reduced cellular response to insulin, arises from specific parts of the intracellular protein interaction network, how this resistance spreads to the rest of the network and even how this resistance may affect other organs [9,10]. This multi-level model is currently being used by several drug development companies to help them identify promising drug targets. Such research on multi-level models has also led to global collaborations and to extensive funding of research networks such as the Physiome project (http://physiomeproject.org/) and the virtual physiological human (VPH, http://www.vph-institute.org/).

Apart from the above-mentioned type of model that spans several layers of biological organization, models can also capture multiple timescales (figure 1). Considering again research on diabetes, there are reported models that not only describe the immediate response to, e.g., a meal or a therapeutic drug treatment, but also describe longer-term dynamics, such as the increase in weight as a result of over-eating, longer-term improvements of a drug, as well as disease development and progression. Some of these models, e.g. those describing immediate effects of a drug—so-called pharmacokinetic and pharmacodynamic (PKPD) models—are today a mainstream tool in the planning of drug certification studies. Yet there also is a trend towards more detailed, mechanistic models, including in pharmaceutical drug research. These more detailed PKPD models are often referred to as quantitative systems pharmacology (QSP) models [11].

Nevertheless, despite such successes, modelling has not yet gained wide acceptance in the biomedical research community. Looking at papers published on diseases such as heart failure and diabetes, an overwhelming majority of them still publishes new data without considering the use of existing quantitative models. Similarly, multi-level models are rarely approved in regulatory decisions to be used as an argument for decision-support in end-usage applications, such as in drug development and for treatment of patients. Even in cases where modelling is used—such as PKPD modelling for the planning and evaluation of drug trials—it is often via the use of oversimplified models, and rarely via the application of the more recent and detailed QSP models. Looking back at founding arguments and beliefs when initiating VPH and other research efforts devoted to multi-level QSP modelling, this slow adoption of modelling may seem unexpected. It is therefore of importance to take a look at the current models and determine the reasons for their lack of broader acceptance and applications. What are the main remaining challenges and how may these be overcome?

We will focus this review on type 2 diabetes (T2D)—a metabolic disease manifested through a non-sufficient control of blood glucose levels. The underlying cause of this non-sufficient control is insulin resistance in normally insulin responding tissues, such as muscle, liver and adipose tissue. In addition, the pancreatic beta cells that normally release insulin are in T2D reduced in mass or function or both. T2D is growing to epidemic proportions as a direct cause of excess food intake with an increasing obese population worldwide. Mathematical models have been used extensively to study different aspects of T2D: a recent comprehensive review of mathematical models that have been developed to understand, diagnose and treat T2D is found in [12]; multi-level modelling in T2D research has been formulated and captured in reviews such as [13,14]; PKPD models in T2D have been reviewed, e.g., in [15] and T2D disease progression models have been reviewed in, e.g., [16]. However, none of these reviews has sought to extract the benefits of bringing these types of models together, nor have they sought to extract the key challenges that still remain before modelling may become more widely adopted in research, in drug discovery and development, in regulatory decisions, and in clinical practice. Herein, we present such an analysis.

We start this review by outlining the state-of-the-art of models in T2D research, ranging from models describing disease-relevant mechanisms and drug response over short timescales, to disease progression models that describe the development of T2D over several years (figure 1). Based on this overview, we identify the most important gaps and weaknesses in existing models. We also go through different approaches of how to model disease progression, and show a new example where an approach with time-varying parameter trajectories is used to predict dynamic changes in the function of beta cells and in insulin sensitivity with anti-diabetic treatments. This small digression from the review format provides an example from the front line of method development within disease progression here applied to T2D data. We then move on towards questions of model integration over multiple timescales, and finally end the review by identifying some of the key challenges that we believe must be overcome before mathematical modelling can be adopted more widely in the overall field of T2D.

### 2. State-of-the-art of mathematical models of type 2 diabetes

The different types of models covered in this first part of the review (divided into three sections) are illustrated in figures 1 and 2. In the first section ('Disease-relevant cellular mechanisms'), we review models that are developed to unravel the cellular origin of disease (figure 2a,c,d); in the second section ('Whole-body disease manifestation and drug effects: short timescales'), we cover the models for disease manifestations
at the whole-body level selected for their relevance in multi-level modelling or for their ability to address differences between patients or both (figure 2). In the third section of this part of the review (‘Disease progression and treatment: long timescales’), we move on to several alternatives for modelling long-term changes over weeks, months and years (figure 3). Finally, note that the brain is not included in the following organ-specific outline (figure 2d). The reason is that available models that cover the brain consider the brain as an alternative control mechanism for whole-body glucose homeostasis. Because these models do not cover intracellular disease mechanisms within neurons and astrocytes, those brain-centred models are covered instead in §2.2.4.

2.1. Disease-relevant cellular mechanisms: short timescales

2.1.1. Insulin resistance mechanisms

In a series of papers [9,10,22,23], the intracellular insulin resistance mechanisms in human adipocytes have been unravelled, with a combination of systematic experimental and mathematical modelling approaches. The experimental data consist of both dynamic time-series (i.e. multiple time-point measurements collected on a timescale of minutes to 1 h) and dose-dependent steady-state data for key signalling proteins in the insulin signalling network. These signalling proteins were measured in primary adipocytes, both from non-diseased and T2D individuals. The data achieved from cells of T2D individuals displayed reduced insulin signalling throughout the network, when compared with data from non-diseased individuals [9,10]. In a mathematical modelling approach, all data could be analysed simultaneously. The analysis revealed three differences between normal and T2D insulin signalling that could explain insulin resistance throughout the network: (i) reduced number of insulin receptors, (ii) reduced number of glucose transporters and (iii) a reduced feedback signal in the signalling network in T2D when compared to the healthy state. While the first two differences (i and ii) have been measured in adipocytes from T2D individuals, the reduced feedback signalling that was identified (iii) was a direct result of the modelling approach. With these differences (i–iii), data from both normal and insulin-resistant T2D individuals could be simulated using the same mathematical model [9,10]. The model can thus be used to simulate the acute intracellular effects of anti-diabetic drugs. Furthermore, this intracellular model has been connected to a whole-body model of glucose–insulin dynamics and with even more detailed models for insulin binding to its receptor [9,22,24]. These insulin signalling models may, therefore, be further used in the study of metabolic evaluation of other drug targets. Prime candidates are cancer drug targets, because insulin signalling proteins are key proteins in cell growth and thus potential therapeutic targets in cancer research. Consequently, for cancer drugs that target intracellular kinases, metabolic effects are unwanted side effects (reviewed in [25]). While the models of insulin resistance [9,10] describe both the normal and T2D state of signalling in adipocytes connected to whole-body effects, they do not contain details of disease progression, i.e. details on the long-term changes that define the transition from normal to insulin resistance. In addition, there are as yet no corresponding models of insulin signalling and insulin resistance for muscle or liver cells (figure 2d).

2.1.2. Liver glucose metabolism

In human hepatocytes, glucose metabolism is under the control of the hormones insulin, glucagon and adrenaline. This metabolism has been modelled using data from multiple clinical studies, on acute glucose response to changes in these hormones [26]. Interestingly, this hepatocyte model has been applied to understand the role of glucose metabolism in the liver during hypoglycaemia, which occasionally occurs in insulin-treated T2D patients [27]. Using the hepatocyte model [26], the authors predict a high risk of hypoglycaemia as related to a reduced response in liver glucose production because of an impaired hormonal control in T2D patients [27]. However, this reduced glucose production by the liver is predicted to be improved following normalization of the plasma glucagon profile. In fact, model-based predictions show an enhanced improvement in liver glucose production with glucagon treatment versus insulin treatment.

2.1.3. Mechanisms of decreased insulin secretion

Mechanisms of insulin secretion have been modelled to understand, e.g., insulin vesicle trafficking, the two phases of insulin release after a meal, and oscillations in the basal insulin secretion pattern (reviewed in [16]). Even though most data from pancreatic beta-cells are taken from animals,
recent models of electrical activity in beta cells have been
developed using human data [28–30], albeit from healthy
individuals only. The lack of models describing the mechan-
isms behind altered insulin secretion in T2D can most likely
be related to difficulties in obtaining data directly from
human pancreas tissue of T2D patients.

2.1.4. Remaining challenges in the modelling of disease-relevant
cellular mechanisms
In T2D research, modelling has been used to some extent to
study disease-relevant cellular mechanisms, especially in
the case of insulin resistance mechanisms in adipocytes.
Such models have been developed in recent years and have
already been integrated over several layers of biological
organization. However, these models have not yet been
used to test different new anti-diabetic drug targets, and
they have not yet been linked to PKPD models for anti-
diabetic drugs to test hypotheses of mechanisms of action
for these drugs. The multi-level model [9,22] so far also
lacks important cellular processes, such as fatty acid uptake
and release, as well as the secretion of and response to hor-
mones other than insulin. When it comes to models of
other organs of interest in T2D research, i.e. muscle, liver
and pancreas, there still are challenges remaining in under-
standing the basic mechanisms behind the disease, and
more data from humans should be collected. Another remain-
ing challenge is to connect the developed short-term models
for acute responses to longer-term disease progression
models, to better understand disease development at the cel-
ular level. It also often is a challenge to re-implement models
from the literature, especially when it comes to under-
standing and evaluating the main underlying assumptions
and data used to build and qualify the models. It is therefore
difficult for a new user to understand the resulting strengths and
weaknesses of the models in existence today [31]. With such
complete information, one would be able to refine, expand
and challenge the models in the light of new data. Finally,
the current multi-level models including disease-relevant
cellular mechanisms are only describing, at this point, an
‘average individual’: an important challenge to overcome,
for these models to reach significant end-usage, is to make
them patient-specific, i.e. calibrated for specific patient
phenotypes, within a given disease context.

2.2. Whole-body disease manifestation and drug
effects: short timescales
2.2.1. The need for mixed-effect models in drug development
We now turn to the case of population models, i.e. models
that describe how the behaviour of particular feature(s)
mechanistic relationships

systems pharmacology model

model prediction of disease progression

comparision with clinical study

Figure 3. A systems pharmacology model translates mechanistic relationships to disease progression and treatment. A featured example of how to integrate different timescales in model development is the model developed in [17]. Therein, short-term cellular data are used to establish relations between (i) the concentration of the inflammatory substance interleukin-1β (IL-1β), (ii) the anti-diabetic drug anakinra which acts as an antagonist at the IL-1 receptor and (iii) replication and apoptosis of beta cells. These relations are combined with a long-term disease progression model, in order to simulate long-term effects of the drug anakinra on beta-cell mass and function and to predict drug effects on HbA1c in a clinical study. (Online version in colour.)
Box 1. ADAPT approach applied to disease progression in type 2 diabetes.

As a base for this example, we use the disease progression model of de Winter et al. [18]. This model describes the dynamics of fasting plasma insulin, fasting plasma glucose and HbA1c under treatment with e.g. the insulin secretion stimulator gliclazide (figure 4).

The model is partly driven by time-varying fractions of beta-cell function (B) and insulin sensitivity (S) which are not determined by the model, but are external inputs to the model [18]. These represent the chronic loss of both beta-cell function and insulin sensitivity and are implemented by letting the coefficients B and S decline as asymptotic functions of time. In addition, the model is driven by the stimulatory effect on beta-cell function by gliclazide treatment (figure 5).

These linear functions can easily be replaced, and different input functions can be chosen as model inputs. In this way, different hypotheses can be tested regarding the progressive changes in beta-cell function and hepatic insulin sensitivity. Instead of prescribing these changes in beta-cell function and hepatic insulin sensitivity—or measuring these parameters using demanding clamp studies—we here infer dynamic trajectories for these biologically and clinically relevant parameters from the available experimental data using ADAPT [19,20]. In this way, the model could serve as an ‘observer system’ integrating and translating biological data into clinically relevant information. The parameter trajectories for fractions of beta-cell function and insulin sensitivity are fitted to the experimental data of fasting plasma insulin, fasting plasma glucose and HbA1c levels by defining B and S as time-dependent parameters, representing simultaneous natural disease progression in combination with the treatment effect (figure 6).

Without predefining how the beta-cell function and insulin sensitivity are expected to change over time, the ADAPT predictions resemble the clinical read-out parameters more closely than the original model. Furthermore, whereas the changes over time in beta-cell function and insulin sensitivity as proposed for the original model [18] are linear over time (despite the assumption that they should decrease in an asymptotic manner), the parameter trajectories inferred by ADAPT show a more dynamic behaviour. The clinically observed data suggest changes in the underlying physiology to occur mostly in the beginning of the study, reaching a plateau after around 200 days. This hypothesis matches the predicted trend in beta-cell function and insulin sensitivity by ADAPT. Presumably, this dynamic behaviour better reflects the physiological changes to different anti-diabetic treatments.
described in the model vary across the population (figure 2b). Such interindividual differences within a population under study are not necessarily described in early phases of drug development (e.g. discovery, preclinical), as it is usually sufficient to know the ‘average effect’ of a compound at those early stages. In contrast, in later stages of drug development (phase 2 and 3), it is no longer sufficient to understand the ‘average effect’ of a compound. Additionally, the interindividual variability distribution become increasingly important to predict, because outstanding individuals may be more at risk of increased toxicity and/or decreased efficacy. Mixed-effect modelling is a standard way of properly taking interindividual variability into account. Here, the parameters of the average population are estimated simultaneously with the variability within the population using all data from all individuals [32]. This simultaneous use of all data separates interindividual variability from residual error, and also allows for more robust estimations in cases of limited data availability from each individual [33]. Furthermore, in mixed-effect modelling, covariates can be included in the model as an effort to explain seemingly random interindividual variability. Mixed-effect models have been used extensively within pharmaceutical research, but often using quasi-phenomenological PKPD models, instead of models built on a mechanistic understanding of the system/disease.

2.2.2. Glucose and insulin interplay
Mathematical models that quantitatively capture the interplay between glucose and insulin within minutes or hours after a meal (figure 1), or following other perturbations, have been developed over several decades. A full account of these models is outside the scope of this review. Nevertheless, some key models must be mentioned. The minimal glucose
model [34] developed in 1979 is still used extensively today—both in research projects and for diagnosis of T2D. For the elucidation of the postprandial insulin response, a hallmark model is the model for meal simulation developed by Dalla Man et al. [35], based on data from more than 200 healthy individuals and a few T2D patients. The same model, but with insulin secretion replaced with external insulin inputs, has also been applied to data from type 1 diabetic patients, and approved by the Food and Drug Administration to be used instead of test animals when testing closed-loops algorithms for insulin administration [4,5]. The model of Dalla Man et al. [35] has been linked to the mechanisms of intracellular insulin signalling [22] (figure 2), using input and output profiles from the whole-body model as constraints in the development of the intracellular model. This multi-level model of Nyman et al. [22], linking whole-body glucose–insulin interplay with intracellular insulin signalling, has also been used in multi-level simulations of insulin resistance in T2D [9]. An alternative model of glucose and insulin dynamics, which also covers interindividual variability, is the integrated glucose–insulin model [36]. This semi-mechanistic model is frequently used to verify drug mechanism of action, quantify drug effect and trial design optimization. The same basic model structure has been adopted to simulate several different glucose provocations both for healthy, impaired glucose tolerance, and T2D patients [37–39]. In addition, the model has been extended with more biomarkers than just glucose and insulin (i.e. glucagon [40], c-peptide (a biomarker for secreted insulin) [41], haemoglobin A1c (HbA1c, a biomarker for long-term glucose) [42]. The diversity of the model combined with the integrative structure enabling prospective simulations and the ability to describe population variability is part of the explanation of the popularity of this integrated glucose–insulin model.

2.2.3. The interplay between glucose and fatty acid homeostasis
Not only is glucose control deregulated in T2D, but also is the control of circulating fatty acids, and these two systems are tightly intertwined. Fatty acids are released from adipose tissue and become involved, through ectopic deposition and metabolism, in the T2D-associated insulin resistance seen in muscle and liver (figure 2d). The acute effect of elevated fatty acid concentrations is reflected in the enhancement of glucose-stimulated insulin secretion. In contrast, the long-term effect is one of the opposite actions, i.e. long-term elevation of fatty acids lowers glucose-stimulated insulin secretion. This situation exemplifies the need for an increased understanding of the different timescales involved in the control of metabolism, and in an understanding of the dysregulations that occur in T2D. Models have been applied to analyse data for postprandial fatty acid fluxes [43] and steady-state relations between glucose and fatty acids in rest and exercise [44,45]. In a more comprehensive approach, Sips et al. [46] used mathematical modelling to translate the information from clamp study data to physiological conditions, and could thus quantify the importance of fatty acids for postprandial glucose control.

2.2.4. Other regulatory systems
Even though most modelling has focused on the interplay between glucose and insulin, with some recent additions of fatty acid homeostasis, dysregulated glucose homeostasis in T2D does, in fact, involve a wide array of interrelated control systems. When it comes to such control systems that have been studied using mathematical models, the selfish-brain theory, as one example, comes to mind, whereby the brain ensures its own glucose supply. This selfish-brain theory has been modelled in a series of papers [47–49], based on experimental data including direct neural stimulation. Another theory about such a control system is known as the set-point theory, and it is described in the last review section below. Apart from the brain control of glucose homeostasis, incretin hormonal effects on insulin secretion have been explicitly studied with mathematical models [50,51]. These models were developed to quantify the incretin effect, i.e. to account for the differences between orally and intravenously administered glucose for different glucose tolerance states. Several other control systems are believed to be important, e.g. inflammatory responses and a wide array of circulating cytokines, but these control systems have not been systematically modelled.

2.2.5. Remaining challenges in the modelling of disease manifestation
In T2D research, plasma concentration profiles of glucose and insulin are commonly analysed with mathematical models. There are also connections between the glucose–insulin models and intracellular models for disease mechanisms (i.e. multi-level or QSP models), at least when it comes to insulin resistance in the adipose tissue. However, these multi-level models have not yet been integrated with the disease progression models (described in the next section) and the multi-level models still only describe an ‘average individual’, i.e. they do not make use of mixed-effect modelling. Multi-level models are, therefore, used primarily in early stages of drug development. Another remaining challenge is to involve more of the regulatory hormones and other regulatory systems that interact with the glucose–insulin interplay, to quantify the relative contributions to glucose control and T2D development, from the different regulatory systems. This quantification is needed both for the not-yet modelled systems, and for the already-modelled selfish-brain system.

2.3. Disease progression and treatment: long timescales
We now turn to the case of long-timescale models, where model features are described over weeks, months or years. These are the timescales over which T2D develops, which also implies that all previously described models cannot simulate the long-term aetiology of T2D, unless they are modified in some way. A common way to modify the models is to introduce slow changes in some of the model parameters. These slow changes can be introduced in the models through different approaches. The most common approach is to use a predefined parameter profile based on assumptions or hypotheses of the changes in these parameters as the disease progresses. Models in this category in T2D research implement hypothetical changes in insulin sensitivity or insulin secretion, or both (see below). Another approach is to experimentally assess the changes in disease parameters. However, experimental assessments are not possible for all mechanistic parameters expressed in short-term models of T2D, but could be used for some specific, well-known disease parameters. Experimental assessments of mechanistic parameters have been used in other areas of systems biology, for example in the modelling of signal transduction [52] and cellular metabolism [53]. A combined
Long-term disease progression in T2D is related to increased insulin resistance and decreased beta-cell mass and/or function (sometimes considered together under the one name ‘functional mass’). De Gaetano et al. [54] have proposed a T2D progression model where an increasing insulin resistance is the primary driver of disease progression. This model is based on long-term interactions among glucose, insulin and beta-cell mass, and is based on the work of Topp et al. [55]. The De Gaetano T2D progression model [54] has been used to simulate observations from the diabetes prevention programme [56]. In another approach, Palmér et al. [17] used the De Gaetano model to simulate the long-term effects of the drug anakinra, an insulin secretion-stimulating agent, using short-term observations of the drug effect on isolated human beta cells. Simulation results were compared with observed clinical effects of the drug anakinra [57,58] (figure 3). The model of long-term glucose, insulin and beta-cell mass developed by Topp et al. [55] has also been extended by Ribbing et al. [59] with a population model that accounts for individual variations in pharmacokinetic parameters and applied to study effects of tesaglitazar treatment in a heterogeneous population. In a more theoretical approach, Graham et al. [60] have linked the Topp et al. disease progression model to mechanistic models of oxidative stress and mitochondrial damage in insulin resistance progression. However, none of these T2D disease progression models includes circulating fatty acids. It will, therefore, be an important future challenge to unravel the long-term contribution of fatty acids in the development of insulin resistance.

### 2.3.2. Time-varying parameters in disease progression—analysis of dynamic adaptations in parameter trajectory

ADAPT is a combination of a data-driven and hypothesis-driven approach to infer parameter trajectories from experimental data that do not directly provide information on the disease parameter of interest [19,20]. These parameter trajectories could be considered as ‘phenotypes’ of the combined effects of disease progression and therapeutic intervention. The ADAPT approach is a descriptive model rather than purely predictive. The essence of this method is that it provides plausible predictions on which underlying pathways might be affected and cause shift in phenotypic transition (disease progression or improvement). These insights could be used as guidelines to further investigate possible mechanistic explanations of the cause of the disease progression. We demonstrate the potential of the ADAPT approach in box 1, where we apply ADAPT to a new example from T2D research. The added value of the analysis of parameter trajectories as dynamic phenotypes has previously been illustrated in preclinical studies of dyslipidaemia associated with metabolic syndrome and liver steatosis [21], where the therapeutic effect of a liver X receptor agonist was analysed, and where associated side effects could be explained.

### 2.3.3. Energy intake and expenditure related to set-point theory

Slow disease progression in T2D has been modelled with a variety of approaches, which we have reviewed. The main difficulty in the modelling of disease progression is about data limitations—both when it comes to long-term studies on disease progression at the population level, and when it comes to the level of variations in disease progression among individuals. The developed models cannot be ‘better’ than the underlying data. In the analysis of drug effects, a remaining challenge is to account for tolerance effects and rebound effects [68] in the developed models, because such effects may have significant contributions to the treatment effect. Finally, to date, there is no multi-level model—which would capture both cellular mechanisms and whole-body manifestations—that also captures the long-term evolution of T2D.

### 3. Outlook: what is needed to bring multi-level systems pharmacology models to end-usage?

Following this overview of T2D state-of-the-art modelling, we now turn to the second aspect of this review: a critical analysis of why modelling, and especially multi-level modelling, has not yet reached widespread end-usage. We will consider three aspects of usage: in research, in drug development and in the clinic (figure 7). A short summary of our claims are as follows: for end-usage in basic research and early-stage drug development, models are indeed ready for usage, as long as there is fast and full access to the models and their underlying assumptions. For end-usage in late-stage of drug development, and especially for an integrated usage that translates across stages of drug development, models must integrate short-term multi-level models with long-term disease progression models, which ideally should also be able to describe variations across a patient population. Finally, for end-usage in the clinic by physicians and patients, multi-level models must—apart from the previous additions—also be embedded.
using multi-level models. For these reasons, the most of all involved subsystems can only be fully unravelled resulting whole-body energy homeostasis. This joint action systems, which, moreover, are acting with small-scale models. This is the case since T2D is a dis-
just remains to be found. Similarly, multi-level models can effectively be rejected, or if an acceptable parameter set
can be understood and pruned to a fit-for-purpose version of the model—and all its data and underlying assumptions—must
be implemented: models must become easily accessible and unambiguously defined. This is necessary, because the
ability to integrate these insights into a complete picture, using the methodologies in, e.g. references [9,22].

Figure 7. The different extensions that are needed to make modelling useful for end-usage. The inner circle describes current multi-level models and cur-
rent modelling methodologies, which are already used for basic research. The next circle describes those additions that are needed to make current multi-level models useful for drug development, including a new type of integration in drug development, whereby information and models follow the drug from the early- to the late-stage phase. The outermost circle describes those additional improvements that are needed to make current multi-level models useful also in the clinic.

3.1. Current models are ready to be used in basic research

Models and modelling methodologies that exist today are ready for usage in basic research, and are already being used to some extent. A prime example is the above-
mentioned unravelling of the origin of insulin resistance in human adipocytes [9,10,22,23]. In these papers, as in many other similar modelling papers, different hypotheses (tenta-
tive mechanistic explanations) already proposed in research literature were translated into mathematical models, and experimental data were then used to distinguish between acceptable and rejected hypotheses. This is usually done in an iterative fashion, where one first checks which models can and cannot explain the available data, and where one then identifies predictions that are useful for experimental design. This iterative process is similar to how experimental research is actually performed, but the model-based analysis enhances the capability of correctly analysing the data. Data analysis without the usage of modelling can lead to incomplete, and sometimes even incorrect, results [70,71].

It is important to note that the properties and potential applications of multi-level models are only partially overlapping with that of smaller-scale models. Small-scale models are good for testing specific hypotheses and for analysing predictions with uncertainty (core predictions), which is criti-
cal in experimental design [72,73]. These tasks are much more difficult to support via large-scale multi-level models, because the parameter space then becomes so large that it is hard or impossible to know whether a model structure can effectively be rejected, or if an acceptable parameter set just remains to be found. Similarly, multi-level models can describe features in a more realistic way, when compared with small-scale models. This is the case since T2D is a dis-
ease that involves several organs, hormones and control systems, which, moreover, are acting together to produce the resulting whole-body energy homeostasis. This joint action of all involved subsystems can only be fully unravelled using multi-level models. For these reasons, the most beneficial use of modelling is first to perform small-scale

3.2. For models to be useful in translational drug development, they must be accessible, personalized and multi-scale

Early-stage drug development is in many aspects similar to basic research, and therefore today's models and method-
ologies can be viewed as sufficiently developed for practical usage. However, for scientific models to be used more widely by pharmaceutical companies, some improvements must be implemented: models must become easily accessible and unambiguously defined. This is necessary, because the time frame of an early-stage drug development project is often in the order of months, and this means that the model—and all its data and underlying assumptions—must
be understood and pruned to a fit-for-purpose version faster than what would be the case in a traditional research project context.

Another important challenge for pharmaceutical companies concerns the workflow of drug development. Knowledge gained with mathematical models in preclinical and early trans-
lational studies are often ignored in later clinical studies, because there is a lack of integration in the steps moving from preclinical to clinical mathematical models. In addition, there is a tradi-
tion to rely on statistical models or simpler phenomenological PKPD models in late-stage clinical drug development. Another issue is that existing multi-level models developed within the academic community are rarely used in drug development within pharmaceutical companies, probably owing to limited time and hands-on resources to understand and fully handle (fit-for-purpose) these more complex models, and in some

models with multi-level biological organization

+ integrated timescales, personalization, and access

+ decision-support and user-friendly interfaces

Clinical end-users doctors/patients

Models and development preclinical to clinical

Basic research biology/physiology

Clinical end-users doctors/patients

Drug development preclinical to clinical

Basic research biology/physiology

Cost-competitive user interfaces (figures 7, 8 and 9).

In a decision-support system, and other scientific disciplines must be engaged to create user-friendly, well-designed and cost-competitive user interfaces (figures 7, 8 and 9).
cases, owing to insufficient collaboration between academia and pharmaceutical companies. This issue leads to models that describe the biological system in a good way, but that are less adapted for usage in drug development. Furthermore, today’s multi-level models find only limited usage in late-stage clinical trials, because they frequently cannot describe either variations across a patient population (patient phenotypes) or long-term changes in disease development and treatment.

Nevertheless, there are a few examples where preclinical and clinical data have been integrated into translational systems pharmacology models. An outstanding example is the model developed by Palmér et al. [17], whereby a long-term T2D disease progression model [54] was extended with short-term cellular data (figure 3). The data from isolated human beta cells contained information on both disease mechanisms and drug intervention effects. Palmér et al. [17] used the developed QSP model to simulate a 13-week treatment with anakinra versus placebo plus a 39-week follow up period, and compared the simulation results with observed clinical effects [57,58]. The developed model predicted an acute increased beta-cell function with anakinra treatment, and a slower increase in beta-cell function [17]. This work provides a new perspective on disease mechanisms and possible mechanisms of drug action within a QSP approach, where short and long timescales are integrated. This approach can be used to investigate clinical questions and translate preclinical data into clinically relevant insights, in T2D as well as in other disease areas.

Figure 8. Embedding of multi-level models in a decision-support setting. This figure illustrates additional extensions needed, for the models to become useful in the clinic, apart from those already needed to make the models useful in drug development (personalization and long-term disease progression). The bottom box outlines the different data types available at patient and population levels. Some of these data enter the mechanistic model (top middle), some enter as covariates in the mixed-effect extension of the mechanistic model (top right). The mechanistic mixed-effect model is used to predict biomarkers that are not observed. These predictions, together with the not-yet modelled biomarkers enter the statistical model (top left). The output of the statistical model is the basis for a decision-support system: what is the most likely diagnosis of the patient, what is the prognosis and what is the optimal treatment?

3.3. For models to reach clinical end-users, they must be embedded in user-friendly decision-support packages

Two additional important aspects are needed, for models to enter into the realm of full usage within the clinic: the modelling itself (figure 8) and concerning the end-usage implementation (figure 9). Concerning the modelling, patient-specific models that can incorporate all available data and convert them into a decision-support result are needed. The arguably most comprehensive way of achieving this conversion is illustrated in figure 8. At the bottom of figure 8, the available data are depicted. Some of these data, such as fasting
insulin, plasma glucose profiles, etc., can be incorporated into mechanistic models as measured states and parameters. These data thus allow for prediction of patient-specific biomarkers. Note that these mechanistic data also include population data, and that population- and patient data can be combined using already existing mixed-effect modelling methodologies [75]. Mixed-effect models can also incorporate so-called covariates, which are data that are not measured states or parameters, but represents other, more loosely defined properties (age, gender, etc.). Finally, there are some types of data that cannot be included as covariates in the mechanistic model, yet they may instead be entered into a statistical modelling framework, such as a Bayesian graphical network [76]. Note that the outcomes of the mechanistic models are new biomarkers, but that the outcome of the statistical model is a complete medical diagnosis, which may be used for disease prognosis, treatment planning, etc.

Once a statistical model of a sufficient quality is in place, the next phase may begin: embedding of that model in a software package—which needs to be cost-effective and useful in practice. This next phase involves the engagement of multiple scientific functions, not usually engaging with systems biology (figure 9). The first step is to engage those functions dealing with data collection, such as biosensor developers [77], medical informatics and electronic healthcare record experts [78] (figure 9, bottom left). Results from the model also need to be represented in a user-friendly and well-designed way [79,80] (figure 9, bottom right). Design is a new and growing research field that not only is concerned with the physical design of graphical user interfaces, but also with the design of the entire treatment process, including all people, services and social structures involved. Design then allows for a bridge to the next level of science: implementation research (how to make a product used in practice?), health economy (is the new product cost-effective?) and social sciences (how will models change social structures in healthcare?) [80,81] (figure 9, middle level). Finally, all of these research fields need to engage with the final end-users and decision-makers: the healthcare providers, the patients, the regulatory decision-makers and the companies that are involved in producing and embracing the final products (figure 9, top cloud).

All in all, it is therefore clear that, while current multi-level models are ready to be used in some end-usages, such as in basic research and early drug development, more work is needed to make modelling useful across the entire value chain, and especially in late-stage drug development and in the clinic. Nevertheless, once the current models have progressed through all the steps described above, it is likely that they will become a superior alternative for all types of clinical end-usages.

### Figures

**Figure 9.** End-usage implementation of decision-support models. The placement of a developed decision-support model (figure 8) within the overall development of new products that are involved in bringing new products into the clinic. The bottom row describes the data which serve as input to the model (left), the model itself (middle), and the representation of model results in a user-friendly and well-designed interface (right). The middle row describes the different disciplines that play a critical role in the assessment of a new product entry into real usage: how will this product interact with the available social structures (left), what does it take to have it accepted for practical usage (middle) and is it cost-effective (right)? The top cloud depicts all of the non-scientific actors that also need to be engaged, before a new product can enter into clinical practice: the companies, the healthcare providers, the patients, the certification agencies, etc.

### References

