A modeling approach for compounds affecting body composition

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Abstract

Body composition and body mass are pivotal clinical endpoints in studies of welfare diseases. We present a combined effort of established and new mathematical models based on rigorous monitoring of energy intake and body mass in mice. Specifically, we parameterize a mechanistic turnover model based on the law of energy conservation coupled to a drug mechanism model. Key model variables are fat-free mass (FFM) and fat mass (FM), governed by energy intake and energy expenditure. An empirical Forbes curve relating FFM to FM was derived experimentally for female C57BL/6 mice. The Forbes curve differs from a previously reported curve for male C57BL/6 mice, and we thoroughly analyse how the choice of Forbes curve impacts model predictions. The drug mechanism function acts on energy intake or energy expenditure, or both. Drug mechanism parameters (two to three parameters) and system parameters (up to six free parameters) could be estimated with good precision (coefficients of variation typically < 20 % and not greater than 40 % in our analyses). Model simulations were done to predict the energy expenditure and fat mass change at different drug provocations in mice. In addition, we simulated body mass and fat mass changes at different drug provocations using a similar model for man. Surprisingly, model simulations indicate that an increase in energy expenditure (e.g. 10 %) was more efficient than an equal lowering of energy intake. Also, the relative change in body mass and fat mass is greater in man than in mouse at the same relative change in either energy intake or energy expenditure. We acknowledge that this assumes the same drug mechanism impact across the two species. A set of recommendations regarding the Forbes curve, vehicle control groups, dual action on energy intake and loss, and translational aspects are discussed. This quantitative approach significantly improves data interpretation, disease system understanding, safety assessment and translation across species.

Keywords: model based drug discovery, obesity, mathematical modeling, body composition, turnover model, Forbes relationship

Introduction

Body composition and body mass are pivotal clinical endpoints in studies of many welfare diseases, such as obesity and diabetes [1, 2]. These biomarkers can also function as safety variables, for instance, in oncology. Besides, body mass is observed in almost all toxicology studies. Understanding the body composition system requires rigorous monitoring of as many of the following variables as possible: energy intake, body mass, fat mass, energy expenditure, and hormonal regulation. Such studies are generally laborious to monitor and costly to run.

However, mechanism-based pharmacodynamic models of energy balances and body composition can significantly improve quantitative data interpretation and disease system understanding. These also lead to increased precision and confidence in quantitative translation across species. In addition, a model-based approach may reduce study cost by influencing experimental design.

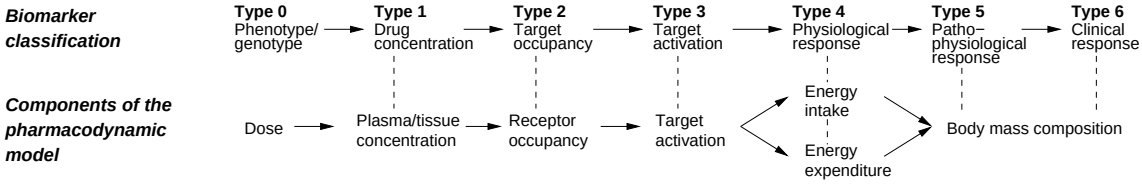

Figure 1: The upper row depicts the general classification of biomarkers [3, 4]. The lower row illustrates key components of the pharmacodynamics model for compounds affecting body composition and/or mass. Dotted lines indicate the relationship between generic biomarkers and model components of the pharmacodynamic model. Particularly, we note that compounds may affect both energy intake and energy expenditure

The aim of this paper is to present pharmacokinetic (PK) and pharmacodynamic (PD) modeling approaches for compounds affecting body composition and body mass. We are currently applying the presented models in drug discovery practice and all approaches are supported by experimental data. The methodologies are generic and not restricted to a specific animal model, even though this particular collection of data is obtained from *in vivo* mouse models.

To exploit a modeling approach, it is useful to consider biomarkers [3, 4] in relation to components of the pharmacodynamics model (Fig. 1). Such a mechanistic overview facilitates communication and reasoning about drug exposure, target binding, and the physiological or disease system on a conceptual level. It is also useful for identification of potential gaps in the data, and division of modeling efforts into subtasks.

This paper is divided into three parts, each describing a fundamental modeling task along the causal pathway that drives body composition changes (Fig. 1). Throughout this analysis, each modeling step is thoroughly described and supported by experimental data.

In Part I, we consider the case where data are sampled for plasma drug exposure and energy intake, and potentially also for target occupancy (type 2) and target activation (type 3) biomarkers (Fig. 1). We simultaneously model vehicle and treatment groups using a relatively simple empirical model with cumulative energy intake as the response variable. The derived models can then be used to generate quantitative predictions of energy intake for various dose or concentration regimens and for prediction of the steady-state concentration–response relationship.

In Part II, we explore models of body composition rather than of specific drug mechanisms. Fundamental to the understanding of body composition is to separately consider energy intake (EI) and energy expenditure (EE). Part II focuses on the common case where temporal data are available for energy intake and body mass, but not for energy expenditure (Fig. 1). Specifically, we take advantage of the semi-mechanistic body composition model proposed by Guo and Hall [5, 6]. Body mass and fat mass are indirectly affected by drugs acting on either energy intake or energy expenditure. We describe how the model is parameterized for our specific diet-induced obesity (DIO) mouse model [7]. The model can then be used to generate quantitative predictions of body composition for various temporal profiles of energy intake and energy expenditure.

We note that Part I and Part II combine drug exposure (Type 1), receptor occupancy (Type 2), and downstream biomarkers (Type 3–4) to relevant endpoints such as body mass (Type 5–6). The main advantages of a complete model include simulation of various dosing schedules, predictions of arbitrary biomarkers along the causal path, and improved understanding of the mechanism of action (e.g. compounds targeting energy intake or energy expenditure, or both).

In Part III, we describe the situation in which data are available for compound exposure and body mass but not for energy intake or energy expenditure (although intermediate biomarkers Type 2 and 3 may be present, as in Part I). In this case we must select a reasonable model for energy intake, which in its simplest form can be a constant intake rate over time. The energy intake model is then coupled to the body composition model of Part II. Part III models can be used to generate quantitative predictions of body composition for various dose or concentration regimens and for prediction of steady-state levels.

The paper ends with a discussion giving concrete advice on experimental design (including a discussion of control groups, number of dose groups, and choice of observation variables), extrapolation over time, and proper choice of modeling approach in different situations.

Table 1: Symbols and abbreviations; first general and then specific for Part I–III of the paper

Name	Unit	Explanation
EI	kcal × day^{-1}	Energy intake
EI_{veh}	kcal × day^{-1}	Energy intake of vehicle group
EI_{drug}	kcal × day^{-1}	Energy intake of treated group
EI_{cum}	kcal	Cumulative energy intake
EE	kcal × day^{-1}	Energy expenditure rate
C	μM	Compound concentration
FM	g	Fat mass
FFM	g	Fat-free (lean) mass
k_a	h^{-1}	Absorption rate
Cl	L×h^{-1}×kg^{-1}	Clearance
V	L×kg^{-1}	Volume of distribution
$I(C)$		Compound inhibitory function
$S(C)$		Compound stimulatory function
I_{max}		Maximum inhibition potential
IC_{50}	μM	Concentration at 50% inhibition
n		Hill exponent/sigmoidicity factor
λ	kcal×g^{-1}	Physical activity
Part I specific		
τ	h	Infusion duration
p_1	kcal×h^{-1}	Initial EI_{veh} in Part I
p_2	h^{-1}	Decline of EI_{veh} in Part I
Part II specific		
ρ_{FFM}	kcal×g^{-1}	Energy density of fat free mass
ρ_{FM}	kcal×g^{-1}	Energy density of fat mass
γ_{FFM}	kcal×g^{-1}×d^{-1}	Metabolic rate of fat free mass
γ_{FM}	kcal×g^{-1}×d^{-1}	Metabolic rate of fat mass
η_{FFM}	kcal×g^{-1}	Synthesis of fat free mass
η_{FM}	kcal×g^{-1}	Synthesis of fat mass
k_i, $s_1 - s_3$		Parameters in $FFM(FM)$ (Eq. 13)
$q_1 - q_3$		Parameters in α (Eq. 14)
K	kcal×g^{-1}	Thermogenesis rate
β		Diet-induced thermogenesis factor
EI_{stand}	kcal×d^{-1}	Energy intake for standard diet compared to high-fat diet
Part III specific		
V_{max}	μM×h^{-1}	Maximum elimination rate
K_M	μM	Michaelis-Menten constant
$r_1 - r_6$		Parameters in EI_{veh} (Eq. 19)

Materials and methods

In order to improve readability, function arguments are not explicitly written out (for example $A(t, p)$ is referred to as A) in cases where the risk of misinterpretation is considered negligible. A list of the main symbols and abbreviations is given in Table 1. All calculations were performed in Matlab (R20112a, The MathWorks, Inc., Natick, Massachusetts, US). The Matlab function ode15s was used for numerical integration, and lsqnonlin was used for non-linear least squares. Appropriate error models were evaluated by visual inspection of residual plots and by comparing parameter precision. In reported fits, a proportional error model was used for PK modeling, and an additive error model was used for PD modeling. Non-linear mixed effects (NLME) models have not been considered in this paper. It is straightforward to extend the models we present to NLME models should one want to perform such analyses.

Models of Part I to Part III are available in a Supplement.

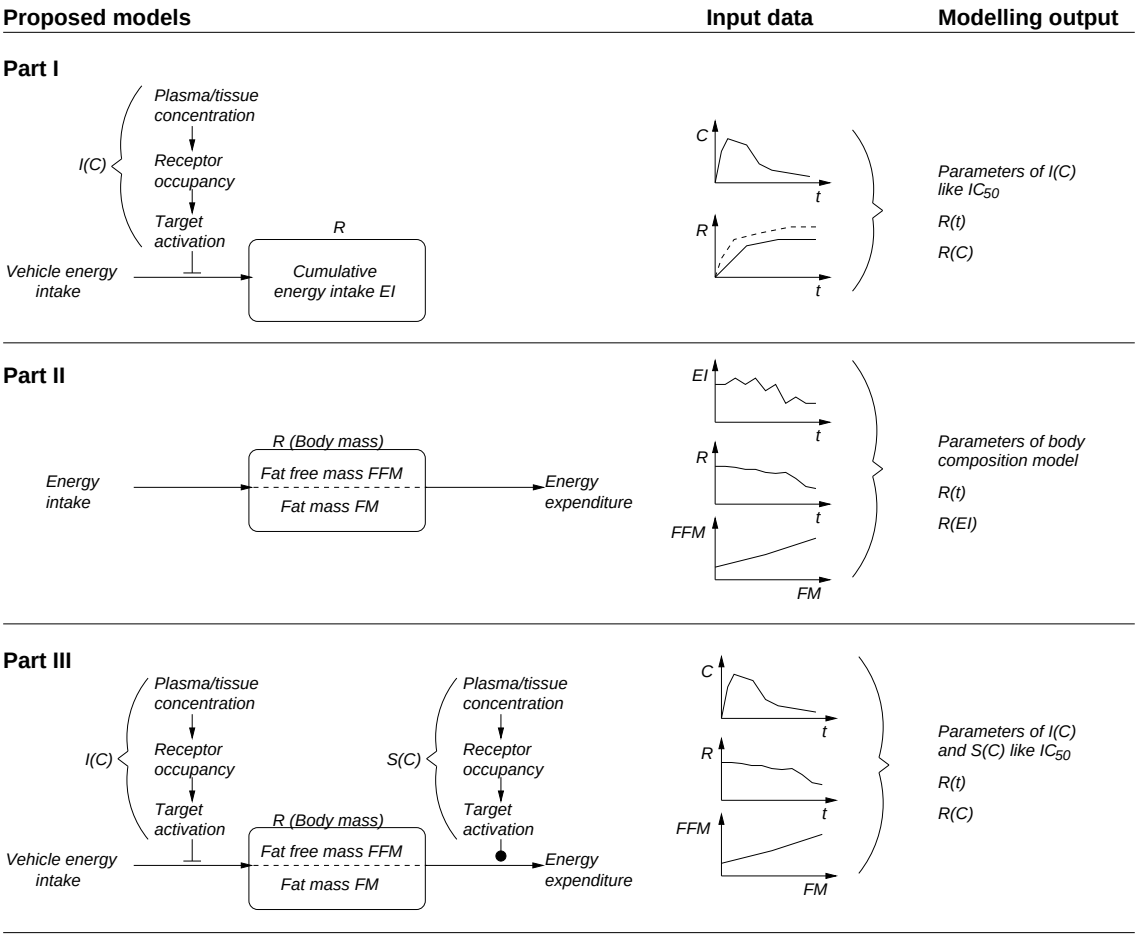

Figure 2: Conceptual models considered in the paper. ***Part I*** *Modeling energy intake. Input data consist of plasma/tissue concentration (and/or target occupancy and/or target activation biomarkers) and cumulative energy intake (dashed line and solid line for control and treated animals, respectively). $I(C)$ (inhibitory action) to the compound mechanisms, inhibiting energy intake.* ***Part II*** *Modeling body composition. Input data consist of energy intake, body mass and an empirical relationship between fat free mass and fat mass.* ***Part III*** *Modeling body mass when energy intake is not measured. Input data consist of plasma/tissue concentration (and/or target occupancy and/or target activation biomarkers), body mass, and an empirical relationship between fat free mass and fat mass as in Part II. $I(C)$ is defined as for Part I, and $S(C)$ (stimulatory action) refers to the compound mechanism activating energy expenditure*

Part I. Modeling energy intake

To model energy intake as a function of exposure and potential downstream biomarkers, we present the basic model in Fig. 2 (Part I). Key assumptions of this approach are that the compound inhibits energy intake rate, and that energy intake rate is limited by that of the vehicle group. The latter assumption is reasonable as long as exposure and target occupancy are sufficiently high to prohibit compensatory feeding at minimum exposure and target occupancy. Sampling of energy intake data is often dense, as in Fig. 3b, where observations are reported hourly. Energy intake is monitored using a food chamber connected to a scale. Occasionally, animals take excess amounts of food which they ingest over several hours. As a consequence, the individual variation in energy intake rate per hour is high. It is therefore recommended to model the cumulative energy intake rather than the energy intake rate.

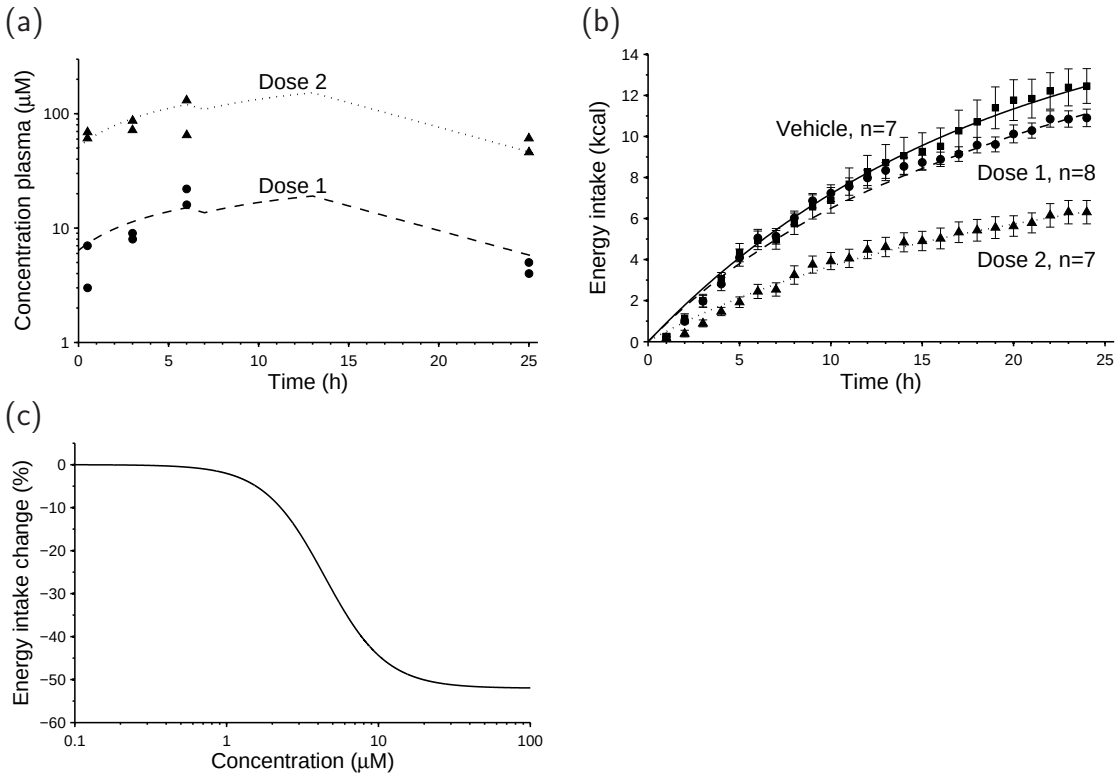

Figure 3: Modeling energy intake (Part I). ***a*** *Exposure data (second treatment day; doses were given twice daily at 0 h and at 7 h) and 1-compartment model fit.* ***b*** *Cumulative energy intake data (standard error of the means are indicated by error bars) and model fit using the model in Eq. 1-3.* ***c*** *Effect* versus *steady-state concentration calculated from the inferred model*

The model is implemented as follows. The time course of plasma exposure to the compound is modeled by standard techniques, as described elsewhere [8]. If target occupancy or target activation biomarkers are available, it is normal to incorporate them in the pharmacodynamic model as an intermediate step (Fig. 2 (Part I)).

Let us then assume that only exposure data are available and that exposure has been modeled as a variable C. The energy intake rate EI_{veh} of the vehicle group is commonly high during the active period of the day (lights off) and low during the resting period (lights on). As a simple example, we can use an exponential decay, defined as

$$EI_{veh} = p_1 \times e^{-p_2 \times t}, \tag{1}$$

with the parameter p_1 representing the initial level and the parameter p_2 representing the monoexponential decline in energy intake. The drug mechanism function $I(C)$ is assumed to inhibit energy intake as

Table 2: Plausible choices of vehicle and compound inhibitory functions that can be combined arbitrarily (there are $3^2 = 9$ possible combinations in the table). The table is not intended to give a complete model space for arbitrary conditions

Energy intake models	Drug mechanism functions	
$p_1 \times e^{-p_2 \times t}$	$1 - I_{max} \times \frac{C}{C+IC_{50}}$	Simple inhibitory function
$p_1 \times t \times e^{-p_2 \times t}$	$1 - I_{max} \times \frac{C^n}{C^n+IC_{50}^n}$	Sigmoid inhibitory function
$p_1 \times \left(e^{-p_2 \times t} - e^{-p_3 \times t}\right)$	$1 - m \times \log(C + a)$	Log linear inhibitory function

$$I(C) = 1 - I_{max} \times \frac{C^n}{C^n + IC_{50}^n}, \tag{2}$$

where I_{max} represents the maximum inhibition potential, IC_{50} corresponds to the concentration at 50 % inhibition, and n is the Hill exponent. The cumulative energy intake rate then becomes

$$\frac{dEI_{cum}}{dt} = EI_{veh} \times I(C), \tag{3}$$

with initial condition

$$EI_{cum}(0) = 0. \tag{4}$$

In this case we assume a direct effect of C on energy intake (i.e. no time delay). This conceptual model can be implemented in different ways by selecting combinations of energy intake functions and drug mechanism functions. The model structure is influenced by the amount and quality of data. A selection of energy intake models and drug mechanism functions is shown in Table 2.

Part II. Modeling body composition

Based on energy intake and body mass data, we will predict body composition and energy expenditure. This part takes advantage of recently developed body composition models that are based on the law of energy conservation and explicitly connected to physiological variables[5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20]. The latter ensures that biophysical constraints (such as conservation of energy) are satisfied and facilitates potential extension of the scope of the models. Input to the model is energy intake and output is energy expenditure. Key model predictions include time series of fat mass, lean mass, body mass, and, in some cases, extra-cellular fluid mass or other variables that are explicitly modeled. Individual models within this class of models mainly differ in the level of resolution at which the system is modeled. Here, we focus on the Guo and Hall semi-mechanistic model for the mouse [5, 6], which is the model species in our *in vivo* studies.

The Guo and Hall model divides body mass into two compartments; a fat compartment with mass denoted FM and a fat-free or lean compartment with mass denoted FFM. The conceptual model is depicted in Fig. 2 (Part II).

The key equation balances energy intake and expenditure as

$$\rho_{FM} \times \frac{dFM}{dt} + \rho_{FFM} \times \frac{dFFM}{dt} = EI - EE, \tag{5}$$

where EI refers to energy intake rate (kcal$\times$day^{-1}), EE refers to energy expenditure rate (kcal$\times$day^{-1}), and the energy densities for changes in FM and FFM are ρ_{FM} and ρ_{FFM}. Here, energy density is defined as amount of energy stored per unit mass (kcal$\times$g^{-1}), and is typically higher for fat mass than for fat-free mass (see also [21]).

Guo and Hall [6] hypothesized that there is a well-defined, time-invariant function, α, that describes the relationship between changes of FFM and FM as

$$\alpha = \frac{\delta FFM}{\delta FM}. \tag{6}$$

This assumption is analogous to the Forbes theory of human body composition change [22, 23]. Originally, Forbes described an empirical, non-linear relationship between fat-free mass and fat mass, using human body composition data, and he hypothesized that longitudinal changes of body composition described this relationship. The functional form of the Forbes curve α is selected based on experimental data from the specific in-house *in vivo* animal model used for body composition modeling.

Given α, the basic model in Eq. 5 can be simplified and rearranged into two ordinary differential equations (ODEs), namely

$$\frac{dFFM}{dt} = \frac{\alpha}{\alpha \times \rho_{FFM} + \rho_{FM}} \left(EI - EE\right), \tag{7}$$

and

$$\frac{dFM}{dt} = \frac{1}{\alpha \times \rho_{FFM} + \rho_{FM}} \left(EI - EE\right), \tag{8}$$

with initial conditions ($FFM(0)$ and $FM(0)$) either as free parameters or, as in this paper, estimated from initial body mass and body-composition data as detailed in the Results section.

In Part II we assume that energy intake, EI, is measured and can be directly input from data. Part III considers the case where energy intake is not measured. Energy expenditure, EE, is modeled as in Guo and Hall (2011).

$$\begin{aligned} EE &= K + \beta \Delta EI + \\ &\quad (\gamma_{FFM} + \lambda) \times FFM + (\gamma_{FM} + \lambda) \times FM + \\ &\quad \eta_{FFM} \frac{dFFM}{dt} + \eta_{FM} \frac{dFM}{dt}, \end{aligned} \tag{9}$$

where K is a thermogenesis parameter (assumed constant for a fixed temperature). Diet-induced thermogenesis corresponds to production of heat that occurs after eating due to ingestion, digestion, and absorption of nutrients as well as transport of blood. It is modeled by the product $\beta \Delta EI$. Here, ΔEI represents the change of energy intake compared to a standard low-fat diet (EI_{stand}; also called chow in Guo and Hall (2011)), and is defined as

$$\Delta EI = EI - EI_{stand}. \tag{10}$$

The parameter $\beta = 0.4$ scales energy intake change to effect on thermogenesis, and has been empirically determined in rodents [24]. Furthermore, the metabolic rate varies with FM and FFM. In the model, it is assumed proportional to FM with constant γ_{FM}, and to FFM with constant γ_{FFM}. The proportional constants γ_{FFM} and γ_{FM} are empirically scaled from human data using the Kleiber 3/4 power law of body mass [25]. The parameter λ (kcal$\times$g^{-1}) represents physical activity (kcal) per gram body mass. Finally, to account for biochemical efficiencies associated with fat and protein synthesis, energy expenditure is assumed proportional to changes in fat mass (proportional constant η_{FM}) and fat-free mass (proportional constant η_{FFM}), respectively [24].

The parameters ρ_{FFM}, ρ_{FM}, γ_{FFM}, γ_{FM}, η_{FFM}, η_{FM}, and β are obtained from the literature. These are then held as constants in the regression of body mass data. In our work, the ΔEI factor of diet-induced thermogenesis (a term in the energy expenditure model) was calculated as the difference in observed EI and prior information on energy intake as 12 kcal$\times$d^{-1} given a standard (not high-fat) diet. For body mass changes in the order of a few percent, K correlates strongly with λ, and identifiability becomes an issue. Therefore, K was assumed fixed at a reasonable value, 2.1 kcal$\times$d^{-1}, taken from Guo and Hall (2011). Model flexibility is still guaranteed by keeping λ a free parameter to be estimated from experimental data. An initial estimate of λ equal to 0.13 was used [6].

To implement the model efficiently, it is useful to algebraically manipulate Eq. 9 in order to remove the derivatives on the right hand side. By inserting Eq. 7 and Eq. 8 into Eq. 9 one obtains a derivative-free expression for energy expenditure as

$$
\begin{aligned}
EE &= (K + \beta \Delta EI + \\
&\quad (\gamma_{FFM} + \lambda) \times FFM + (\gamma_{FM} + \lambda) \times FM + \\
&\quad \eta_{FFM} \times \alpha \times g \times EI + \eta_{FM} \times g \times EI)/ \\
&\quad (1 + \eta_{FM} \times g + \eta_{FFM} \times \alpha \times g),
\end{aligned} \tag{11}
$$

where

$$
g = \frac{1}{\alpha \times \rho_{FFM} + \rho_{FM}}. \tag{12}
$$

We generated data to provide the empirical function α (Forbes curve, relating changes in FFM and FM) for our in-house mouse strain and environment. Briefly, whole body composition (fat and lean mass) was assessed in eighteen female C57BL/6 mice on a high-fat diet at four time points, when average body masses in the population were 20 g, 35 g, 40 g, and 45 g.

The functional form of α is assumed to be the same as the one employed in Guo and Hall [5, 6]. Hence, the following model was used

$$
FFM = k_i + s_1 \times FM + s_2 \times e^{s_3 \times FM}. \tag{13}
$$

The k_i parameter is specific to each mouse (for our data set $i \in [1 \dots 18]$), while the parameters s_1–s_3 are the same for all mice. In total, there are 21 (18 k_i's, s_1–s_3) parameters to estimate. The function α was obtained by taking the derivative of Eq. 13 with respect to FM as

$$
\begin{aligned}
\alpha = \frac{\delta FFM}{\delta FM} &= s_1 + s_2 \times s_3 \times e^{s_3 \times FM} = \\
&= q_1 + q_2 \times e^{q_3 \times FM},
\end{aligned} \tag{14}
$$

where the q_1, q_2, and q_3 parameters represent an alternative parameterization ($q_1 = s_1$, $q_2 = s_2 \times s_3$, and $q_3 = s_3$) that will occasionally be used for convenience.

We compared our results with similar data from male mice of the same strain [5, 26]. Table 3 gives an overview of the experimental settings for the two studies.

Part III. Modeling body mass when energy intake is not measured

Observing body mass requires significantly fewer resources than observing energy intake. Therefore, observing exposure and body mass is more common in studies that take place over days or weeks rather than hours.

To model body mass as a function of exposure (or receptor occupancy or target activation biomarkers), we may consider the conceptual model in Fig. 2 (Part III). Key assumptions of this modeling approach include:

- The compound affects energy intake rate or energy expenditure, or both in the *in vivo* model.
- An *in vivo* body composition model (including the empirical Forbes curve) has been identified (see Part II).

The model is implemented as follows. As in Part I, the pharmacokinetics of the compound is modeled by standard methods. Since body mass is usually observed on a day-to-day basis, it is standard procedure to select a single PK measure for each day (e.g. the average, minimum, or maximum steady-state concentration, $C_{ss,average}$, $C_{ss,min}$, or $C_{ss,max}$, respectively). A more detailed modeling approach is required if the exposure profile fluctuates heavily, if various dosing schedules are applied, or if highly nonlinear PK prevails.

The vehicle energy intake can be assumed constant as a first approximation with time frames in the order of a few days. For studies over several weeks, increasing energy intake is required to supply the increasing body mass, and as a standard approach we consider a linear model defined as

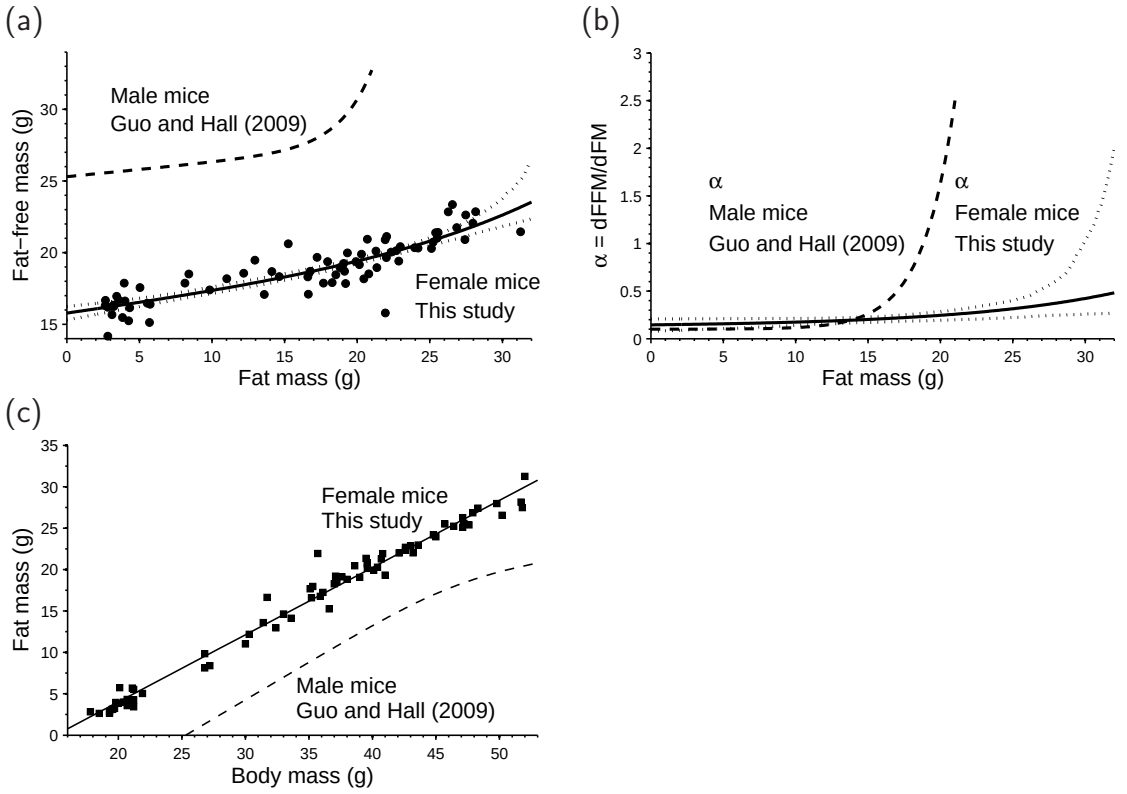

Figure 4: Body composition data for 18 C57Bl/6 female mice (Part II), and comparison to Guo and Hall (2009) (see Table 3 for a comparison of study setups). ***a*** *Data and model fit (Eq. 13) using the parameters s_1–s_3 and the mean of the 18 k_i parameters (solid line; dotted lines indicate 5% and 95% percentiles (obtained by bootstrapping) of the mean curve). Instead of plotting one curve for each mouse, the mean curve is plotted, and, for each mouse, data have been shifted by the individually identified parameter k_i.* ***b*** *The Forbes function α,* i.e., *$\delta FFM/\delta FM$, obtained in this study (dotted lines indicate 5% and 95% percentiles, respectively) and in Guo and Hall (2009). Curves are plotted for the fat mass range covered by data in respective study (up to 32 g and 21 g, respectively).* ***c****. As an initial condition, fat mass can be empirically estimated from body mass (when body mass is in the observational range of 18 g to 52 g) by assuming a linear relationship*

Table 3: Comparison of body composition experiments. In this study, mice had access to two food sources, chocolate pastry and high-fat diet. In Guo et al. (2009) and Guo and Hall (2009), five groups with different diets were considered, and all data were simultaneously analysed. All mice were initially fed with standard diet, and then the following groups (6 mice per group) were considered: (1) standard diet, (2) high fat diet, (3) first high fat diet then a switch to standard diet, (4) high fat diet+liquid ensure, and (5) first high fat diet+liquid ensure then a switch to standard diet. Carb. = Carbohydrate

Variable or parameter	This study		Guo et al. (2009) and Guo and Hall (2009)	
Strain	C57BL/6		C57BL/6	
Sex	Female		Male	
Temperature	18-22 °C		21-22 °C	
Light-dark cycle	12:12 h		12:12 h	
Diets included	Chocolate pastry 4.8 kcal×g^{-1}	Protein 4.7 kcal% Carb. 40.8 kcal% Fat 54.5 kcal%	Standard (NIH-07)	Protein 24 kcal% Carb. 64 kcal% Fat 12 kcal%
	High fat (D12492) 5.24 kcal×g^{-1}	Protein 20 kcal% Carb. 20 kcal% Fat 60 kcal%	High fat (F3282)	Protein 14 kcal% Carb. 27 kcal% Fat 59 kcal%
			Liquid ensure	Protein 14 kcal% Carb. 64 kcal% Fat 22 kcal%
k (mean) in Eq. 13	15.6		25.3	
s_1 in Eq. 13	0.13		0.10	
s_2 in Eq. 13	0.22		0.00042	
s_3 in Eq. 13	0.090		0.45	
q_1 in Eq. 13	0.13		0.10	
q_2 in Eq. 13	0.020		1.9×10^{-4}	
q_3 in Eq. 13	0.090		0.45	

$$EI_{veh} = r_1 + r_2 \times BM \tag{15}$$

with the two parameters r_1 and r_2.

However, in practice, the energy intake of the vehicle group exhibits a significant drop modeled by V_1 when treatment starts, presumably due to handling stress. We let V_1 take the form of an exponentially decreasing additive effect on EI defined as

$$V_1 = \begin{cases} r_3 \times t_{tr} \times e^{-r_4 \times t_{tr}}, & \text{if } t_{tr} \geq 0 \\ 0, & \text{otherwise} \end{cases} \tag{16}$$

where t_{tr} refers to time after treatment start, and where r_3 and r_4 are parameters reflecting the magnitude and first-order decay rate, respectively. In addition, an increased energy intake, modeled by V_2, is typically observed when treatment stops. We assume that V_2 is dependent on the body mass decrease during treatment, defined as

$$\Delta BM = \frac{BM_{\text{tr. start}} - BM_{\text{tr. end}}}{BM_{\text{tr. start}}}. \tag{17}$$

We further assume that V_2 is temporary and exponentially decaying. We model this empirically by

$$V_2 = \begin{cases} r_5 \times \Delta BM^{r_6} \times t_e \times e^{-r_4 \times t_e}, & \text{if } t_e \geq 0 \\ 0, & \text{otherwise} \end{cases} \tag{18}$$

where t_e refers to time after treatment stop, r_5 is a magnitude parameter, r_6 a scaling parameter, and the decay rate (r_4) is assumed to be the same as in Eq. 16. Taken together, EI_{veh} is modeled as

$$EI_{veh} = r_1 + r_2 \times BM - V_1 + V_2 \tag{19}$$

where the first term r_1 represents baseline energy intake, the second term $r_2 \times BM$ growth in energy intake due to growth in size, the third term V_1 a temporal decrease in energy intake due to handling stress at start of treatment, and the final term V_2 represents a temporal increase in energy intake due to cessation of treatment.

The compound may inhibit energy intake by the drug mechanism function $I(C)$ (Eq. 2). The energy intake rate of the group treated with the test compound is then obtained as

$$EI_{drug} = EI_{veh} \times I(C) \tag{20}$$

where EI_{drug} refers to the energy intake (rate) after drug intervention, which is the input variable to the body composition model (Fig. 2 (Part III)). Similarly, the compound may affect energy expenditure EE (Eq. 11), which can be redefined as

$$EE_{drug} = EE \times S(C), \tag{21}$$

where $S(C)$ is a stimulatory drug-mechanism function.

Again, the conceptual model can be implemented in a number of ways by selecting combinations of feasible functions for energy intake and compound inhibitory or stimulatory effect (see Table 2 for a selection of models).

The basic modeling approach is as follows: First, select a vehicle model (Eq. 19 is one example). Second, fit the model to vehicle data only. Free parameters are those of the EI_{veh} model ($r_1 - r_6$). In the third step, select the drug mechanism functions $I(C)$ or $S(C)$ that influence energy intake and those that influence energy expenditure (Fig. 2 (Part III)). Fit the model to treated data only, keeping the vehicle model fixed. As a potential final step, fit the model to vehicle and test-compound-derived data simultaneously. In general, model selection must be iteratively revised.

Animals and housing

Ninety-two female and male C57BL/6 mice were used in these studies. On arrival, the mice were housed in groups of maximum six in Macrolon 2L cages (Scanbur, Karslunde, Denmark) containing aspen wood chip bedding and an enriched environment with free access to food and water. The animal room had a regulated temperature (18-22 °C), humidity (∼50%), and a 12:12-hour light-dark cycle. The mice were allowed at least one week of acclimatization before study initiation. To induce obesity, mice were assigned to a high-caloric diet (chocolate confectionery with 54% kcal as fat: Delicato Bakverk AB, Kungens Kurva, Stockholm, Sweden; and D12492 diet, 60% kcal as fat: Research Diets, New Jersey, USA) at age of 8–10 weeks. Chocolate confectionery was excluded for technical reasons in studies where food intake was measured automatically (Experiments 1, 3, and 4). The local Animal Research Ethics Board Committee (Göteborg) approved these studies.

Part I. Energy intake (Experiment 1, Fig. 3). Twenty-two lean female mice were given a high-fat diet (D12492) on the day of arrival. Eight days later, the animals were housed individually for seven days prior to the start of the experiment for acclimatization purposes. Their food intake was monitored using an in-house automatic monitoring system (AstraZeneca, R&D, Mölndal, Sweden) allowing continuous measurement of food intake in undisturbed animals housed in their home cage environment. After two days of basal food intake registration, the animals (body mass 24.6 ± 0.78 g) were subcutaneously dosed twice daily (t = 0 h and t = 7 h) for two days during the dark phase. Food intake registration was performed during the whole period of the experiment. In the morning of the third day, two animals from each dosing group were terminated and blood collected for plasma substance content analysis (17 hours after last dose). In parallel, the remaining animals from the dosing groups were then dosed a fifth time and plasma collected after 0.5, 3, and 6 hours for plasma compound exposure.

Part II. Body composition (Experiment 2, Fig. 4). Eighteen female mice were given access to a high-caloric diet (D12492 diet and chocolate confectionery) when average body weight exceeded 20 g. Whole-body

composition (fat and lean mass) was assessed in all mice using dual energy X-ray absorptiometry (DEXA) scan (PIXImus2; Lunar, Madison, WI) at four time points, when average body weights in the population were 20 g, 35 g, 40 g, and 45 g, respectively. Each mouse was anesthetized for the duration of the procedure (5 min) by exposure to Isoflurane-oxygen gas via nose cone. Each mouse was placed on the scanner bed in the prone position, with the limbs and tail stretched away from the body. After the scan was completed the mouse was removed from the scanner bed and returned to its home cage to recover from anesthesia.

Part II. Energy intake and body mass, first study (Experiment 3, Fig. 5). To induce obesity, five female mice were placed on a high-fat diet (D12492) for 25 weeks. Thereafter, mice were either administered compound known to affect appetite (n=3) or a vehicle (n=2) by gastric gavage (5 ml $\times$ kg^{-1}) twice daily during the dark phase for 25 days. Body weight was measured daily. Food intake was recorded automatically as described above (Experiment 1). In the data set, five energy intake measures (of in total 125 data points) were missing; these were replaced by the mean of the two neighboring points.

Part II. Energy intake and body mass, second study (Experiment 4, Fig. 6). After three months on a high-fat diet (D12492), 12 male mice were divided into two groups and administered either compound (n=6) or vehicle (n=6) at the beginning of the dark phase for 10 days. Body weight was measured daily. Food intake was recorded automatically as described above (Experiment 1). Data for two of the animals (one from each group) were removed from the study because they were incorrectly administrated for one of the days. The same study was repeated two weeks later, and pooled data from all animals (compound-treated, n=5+6=11; vehicle, n=5+6=11) were used in the analysis.

Part III. Body mass (Experiment 5, Fig. 8). Twenty-three female mice were fed a high-caloric diet (D12492 and chocolate confectionery) for 18 weeks to induce obesity. Thereafter, the mice were randomized into four groups (n=5–6 per group) and administered 1, 10, or 40 μmol $\times$ kg^{-1} of a compound known to affect appetite or a vehicle by gastric gavage (5 ml $\times$ kg^{-1}) twice daily during the dark phase for 21 days. Body weights were recorded daily during the experiment. After the last treatment day, the groups treated with 1 and 40 μmol $\times$ kg^{-1} of the compound were terminated for subsequent compound related analyses. For the control group and the group treated with 10 μmol $\times$ kg^{-1} of the compound there was a washout period of 35 days, during which body weight was measured regularly.

Results

Part I. Modeling energy intake

Monitoring energy intake over one or a few days is a fast, relatively cheap, and often accurate method for judging the ability of a compound to affect body composition chronically [7].

We exemplify the use of the basic model proposed in Fig. 2 (Part I) using real drug discovery data sampled for exposure and energy intake in a lead optimization mice screen, with a test compound that targets a receptor that down-regulates appetite. The compound was subcutaneously administered several days before sampling, as well as on the sampling day (twice daily at $t = 0$ h and $t = 7$ h). The exposure to test compound was modeled by a one-compartment model with zero-order absorption (duration τ), and first-order elimination. Observed and model-predicted data are shown in Fig. 3a. The exposure was fixed and used to drive the inhibitory drug mechanism function (Eq. 1-3). The proposed PD model was simultaneously fitted to all time courses for pharmacological response. For cumulative energy intake data, we note that the choice of error model is complicated due to the summation of daily intake and unknown statistical dependencies between days. For this data set, we found that an additive error model was most appropriate. The obtained parameters are given in Table 4. The time courses for observed and predicted response (cumulative energy intake) are shown in Fig. 3b.

The relatively simple vehicle-group model of energy intake adequately captures major trends in data and has good parameter precision. Based on the model, the relationship between steady-state concentration and response can be generated (Fig. 3c). We will return later to predicting the corresponding relationship between exposure and body mass.

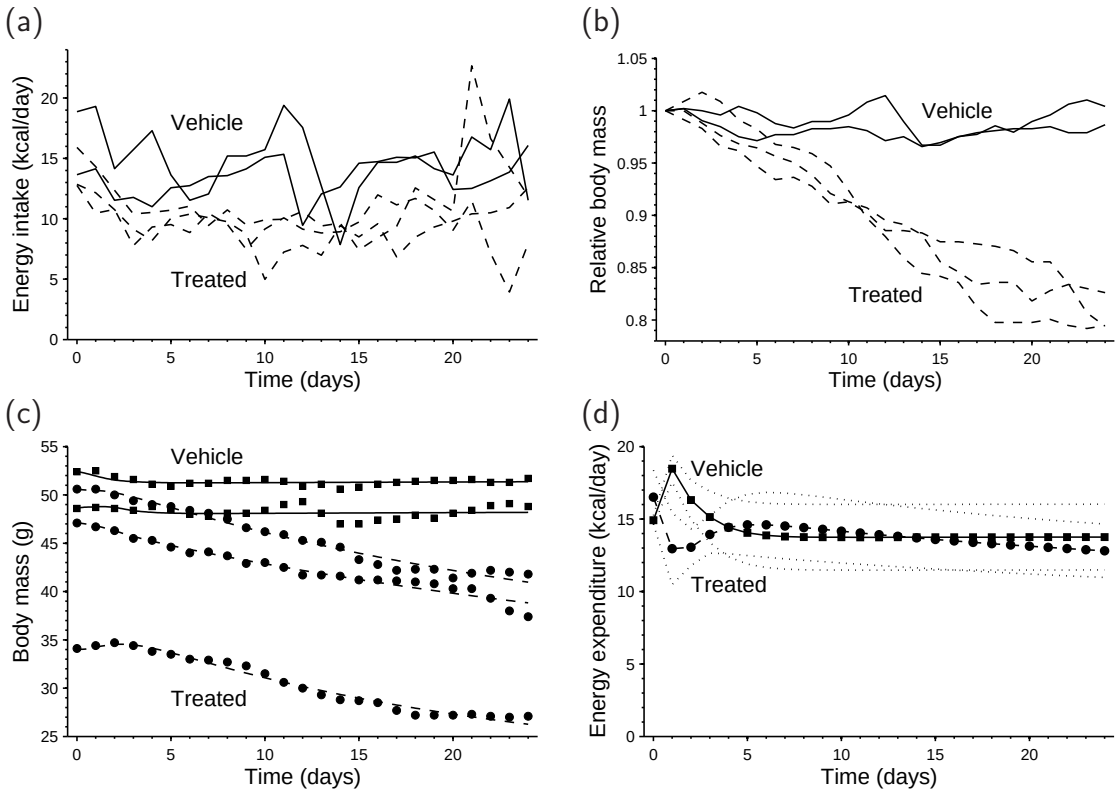

Figure 5: Modeling body composition (Part II, Example 1). ***a*** *Energy intake (EI) data for two vehicle treated mice, and three mice treated with a compound down regulating appetite.* ***b*** *Relative body mass (BM) data for both groups.* ***c*** *Using energy intake data as input the model was fitted to BM data, individually for each animal.* ***d*** *Model based predicted energy expenditure of the vehicle and the treated groups, respectively (means indicated by solid lines and standard error of the means by dotted lines)*

Table 4: Parameter estimates and their relative standard deviation (CV%) obtained from fitting Equations 1-3 to exposure and energy intake data in Part I

Parameter	Unit	Estimate	CV%
τ	h	6.00	29.
Cl	$\mathrm{L}\times\mathrm{h}^{-1}\times\mathrm{kg}^{-1}$	0.0259	12.
V	$\mathrm{L}\times\mathrm{kg}^{-1}$	0.262	36.
p_1	$\mathrm{kcal}\times\mathrm{h}^{-1}$	0.939	2.6
p_2	h^{-1}	0.0555	6.1
I_{max}		0.520	2.8
IC_{50}	μM	4.42	1.5
n		2.15	4.2

Table 5: Parameter values of the body composition model (Part II, Example 1). For λ, an individual value was identified for each mouse

Parameter	Unit	Description	Value	CV%	Reference
ρ_{FFM}	kcal×g^{-1}	Energy density	1.8	fixed	[6]
ρ_{FM}	kcal×g^{-1}	Energy density	9.4	fixed	[6]
γ_{FFM}	kcal×g^{-1}×d^{-1}	Metabolic rate	0.03	fixed	[6]
γ_{FM}	kcal×g^{-1}×d^{-1}	Metabolic rate	0.15	fixed	[6]
η_{FFM}	kcal×g^{-1}	Synthesis	0.23	fixed	[6]
η_{FM}	kcal×g^{-1}	Synthesis	0.18	fixed	[6]
q_1		Parameter in α	0.13	fixed	Data in Fig. 4a
q_2		Parameter in α	0.020	fixed	Data in Fig. 4a
q_3		Parameter in α	0.090	fixed	Data in Fig. 4a
K	kcal×g^{-1}	Thermogenesis rate	2.1	fixed	[6]
β		parameter in diet-induced thermogenesis	0.4	fixed	[6]
EI_{stand}	kcal×d^{-1}	Energy intake for standard diet	10	fixed	In-house data
λ_0	kcal×g^{-1}	Physical activity, veh. animal 1	0.12	2.9	Data in Fig. 5a-b
λ_1	kcal×g^{-1}		0.26	20	Data in Fig. 5a-b
λ_0	kcal×g^{-1}	Physical activity, veh. animal 2	0.16	0.96	Data in Fig. 5a-b
λ_1	kcal×g^{-1}		0.12	21	Data in Fig. 5a-b
λ_0	kcal×g^{-1}	Physical activity, tr. animal 3	0.21	1.5	Data in Fig. 5a-b
λ_1	kcal×g^{-1}		-0.11	40	Data in Fig. 5a-b
λ_0	kcal×g^{-1}	Physical activity, tr. animal 4	0.15	2.2	Data in Fig. 5a-b
λ_1	kcal×g^{-1}		0.11	44	Data in Fig. 5a-b
λ_0	kcal×g^{-1}	Physical activity, tr. animal 5	0.36	1.2	Data in Fig. 5a-b
λ_1	kcal×g^{-1}		-0.70	9.0	Data in Fig. 5a-b

Part II. Modeling body composition

We generated body-fat and body-mass data in order to characterize our specific mouse strain and environment by the empirical Forbes relationship α, which relates changes in FFM to changes in FM (Fig. 4a). To this end, Eq. 13 was fitted to the data (Fig. 4a, Table 3). The resulting behavior of α was obtained as Eq. 14 (Fig. 4b, Table 3). Naturally, the curve depends on strain, environment, food supply, as well on the selected functional form (Table 3). To illustrate this point, the corresponding fits for data obtained in C57BL/6 males reported by Guo and Hall (2009) are shown together with data from this study (Fig. 4a–b, Table 3).

Using the same data set, we established empirical expressions for the initial conditions, such as $FM(0)$ and $FFM(0)$. Based upon the data in Fig. 4c, and by assuming a linear relationship, the initial condition for fat mass $FM(0)$ was obtained from initially observed body mass $BM(0)$ as

$$FM(0) = -12.2 + 0.81 \times BM(0), \tag{22}$$

The initial fat-free mass $FFM(0)$ then follows directly from the two-compartment assumption as

$$FFM(0) = BM(0) - FM(0). \tag{23}$$

The initial fat-free mass $FFM(0)$ for male mice has a different pattern, as indicated in Fig. 4c. The male equation for $FFM(0)$ is implicitly defined by the parameters k and s_1–s_3 (Table 3) in Guo and Hall (2009) (Fig. 4c).

The body composition model is composed of the two fundamental energy equations Eq. 7 and Eq. 8, complemented by the energy expenditure model (Eq. 11 and Eq. 12), as well as by the empirical Forbes curve, which relates changes in fat and fat-free masses (Eq. 14). Initial conditions are obtained from Eq. 22 and Eq. 23.

Example 1

In the next step, we demonstrate how the body composition model can be used in drug discovery. We consider a 24-day study using five mice that had been on high-fat diet for six months before study start.

We utilize energy-intake and body-mass data from three mice treated with a compound targeting appetite, as well as from two control mice (Figures 5a and b). Treatment started on the first day and continued throughout the study. Clearly, data indicate reduced energy intake as well as body-mass reduction for the treated animals.

The model was fitted to body-mass data individually for each animal, using energy intake data as input. In the data-fitting, flexibility of the energy expenditure model was established by a physical activity function λ with two free parameters. The function was defined as

$$\lambda = \lambda_0 + \lambda_1 \times t \times \exp(-t), \tag{24}$$

where the parameter λ_0 represents a basal level, and the second term represent a transient (stress) response to dosing with λ_1 as a parameter.

Hence, for each animal there are two free parameters; λ_0 and λ_1. Model fits are depicted in Fig. 5c, and parameters are detailed in Table 5. We note that the model operates over a relatively large range of initial masses (Fig. 5c).

The identified models predict energy expenditure of the vehicle and the test compound treated groups, respectively (Fig. 5d). The physical activity parameter λ is generally higher in the test compound treated group than in the vehicle group (Table 5). In isolation, this would cause a higher energy expenditure in the treated group. However, energy expenditure is influenced by additional variables (Eq. 9). In particular, the vehicle group has a higher energy intake, and consequently higher diet-induced thermogenesis and less protein and fat degradation, compared to the treated group. In this case, these effects roughly cancel each other, and data indicate no strong compound effect on energy expenditure.

Example 2

As a second example, we consider a 14-day study using 22 mice that had been on high-fat diet for three months before study start. We utilize data from 11 mice treated with a compound affecting appetite, as well as 11 control mice (Figures 6a and b; Table 6). Treatment started on day 3 and ended on day 12. These data also indicate reduced energy intake and body mass for the treated group.

The mice in this study are males, calling for caution as α and $FM(0)$ used in the first example were estimated from data gathered from female mice (Eq. 14 and Eq. 22, respectively). There may be large differences in the relationship between FFM and FM, as clearly indicated in Fig. 4. Therefore, in this case we used α and $FM(0)$ for male mice derived by Guo and Hall [6]. Notably, the functional form of α for male mice indicates that fat mass never exceeds a threshold of about 20 g for a realistic range of body masses.

Again, the model was fitted to body-mass data individually for each animal, using energy intake data as input. In the data-fitting, flexibility of the energy expenditure model was established by defining one value for physical activity λ, referred to as λ_1, during the acclimatization and washout periods (days 0–2 and 13–14), and another value for λ, referred to as λ_2, during the treatment phase (days 3–12). Two representative fits, one from the vehicle group and one from the test-compound-treated group, are depicted in Fig. 6c. Parameters are detailed in Table 6.

In contrast to the first example, the data indicate a compound effect on energy expenditure (Fig. 6d). The final drop in energy expenditure for the treated group is relatively strong, and calls for follow-up studies. For two of the treated animals (number 17 and 20), the model is not flexible enough to capture these drastic changes, which is reflected in high CV's for λ_1. For instance, a longer washout period would reveal whether treated animals regain appetite, energy expenditure, and body mass, or whether the drop in energy intake and expenditure indicates that the treated animals are persistently affected by the compound.

Generating rules of thumb

The body composition model parameterized here can be used to reason about energy-intake and body-mass studies. To facilitate interpretation and stimulate discussions about such studies, we generated a set of rules of thumb for the diet-induced obesity model discussed in this paper (Fig. 7).

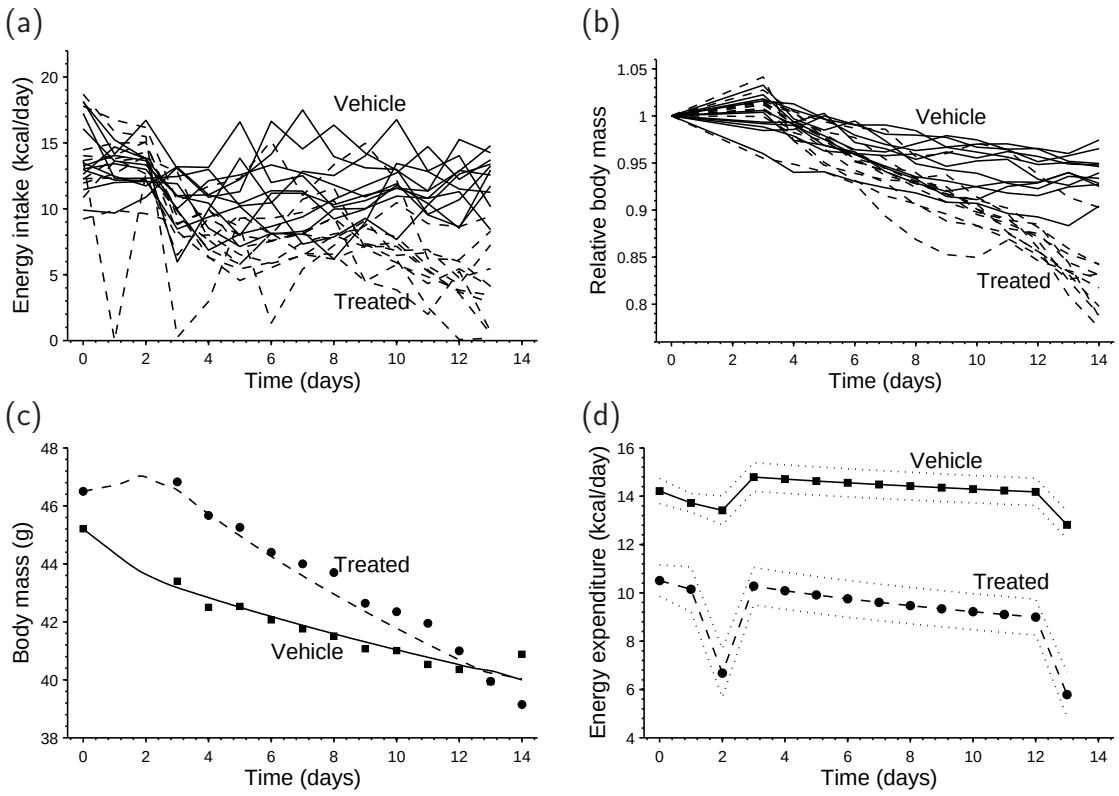

Figure 6: Modeling body composition (Part II, Example 2) ***a*** *Energy intake (EI) data for 11 vehicle treated mice, and 11 mice treated with a compound down regulating appetite.* ***b*** *Relative body mass (BM) data for both groups.* ***c*** *Using energy intake data as input the model was fitted to BM data, individually for each animal. Two representative fits, one from each group, are depicted here.* ***d*** *Model based predicted energy expenditure of the vehicle and the treated groups, respectively (means indicated by solid lines and standard error of the means by dotted lines)*

Table 6: Parameter values of the body composition model (Part II, Example 2). For each mouse, the physical activity parameters λ_1 and λ_2 were identified

Animal	λ_1		λ_2	
	Estimate	CV %	Estimate	CV %
vehicle 1	0.220	5.5	0.200	3.1
vehicle 2	0.0995	7.6	0.175	2.2
vehicle 3	0.113	9.3	0.105	4.6
vehicle 4	0.108	8.7	0.133	4.2
vehicle 5	0.140	11	0.165	4.4
vehicle 6	0.156	5.4	0.152	2.7
vehicle 7	0.146	9.3	0.202	3.5
vehicle 8	0.145	12.4	0.188	5.0
vehicle 9	0.163	6.3	0.192	2.7
vehicle 10	0.140	14	0.158	6.6
vehicle 11	0.128	16	0.263	3.5
treated 12	0.0644	20	0.126	5.7
treated 13	0.0857	8.6	0.185	2.3
treated 14	0.0595	48	0.0967	16
treated 15	0.0385	36	0.133	6.3
treated 16	0.0491	13	0.134	3.0
treated 17	0.0141	>100	0.101	17
treated 18	0.0808	19	0.128	6.5
treated 19	0.220	10	0.240	5.1
treated 20	0.00258	>100	0.132	16
treated 21	0.0572	28	0.161	5.5
treated 22	0.0241	50	0.143	4.9

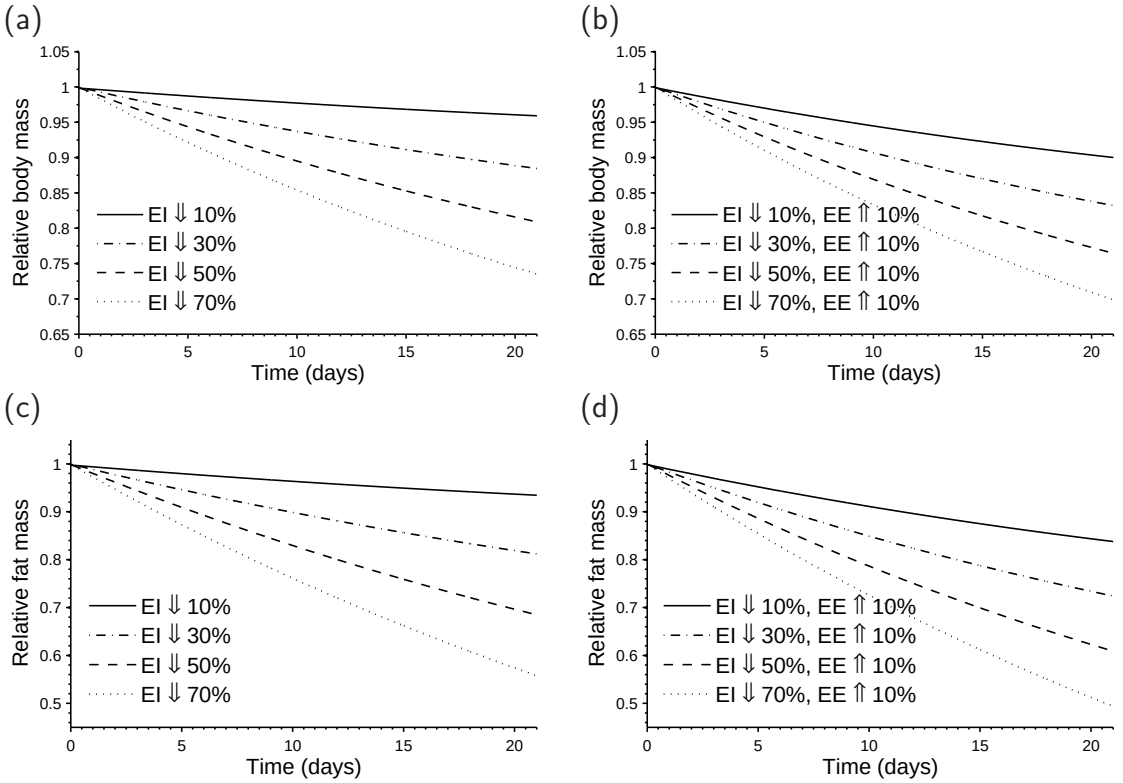

*Figure 7: Rules-of-thumb for body mass changes. Body mass change given no effect on energy expenditure (**a**), and assuming 10% treatment effect on energy expenditure (**b**). Fat mass change given no effect on energy expenditure (**c**), and assuming 10% treatment effect on energy expenditure (**d**)*

Part III. Modeling body mass when energy intake is not measured

Modeling body mass when energy intake is not measured is difficult when data or prior information, or both, are limited. In such cases, standard turnover models could be used.

Here, we utilize experimental data from a study with several dose groups and with an extensive washout period (Fig. 8a). Three dose groups were treated with an appetite-regulating compound, and one additional group served as a control. Each group consisted of 5–6 animals. Treatment started on day 0 and ended on day 20. The animals in two of the groups were monitored up to day 56 (although the compound is expected to be cleared within one day).

The exposure to test compound was captured by a one-compartment model with linear absorption and Michaelis–Menten elimination. The absorption rate constant k_a was fixed (1 h^{-1}) in the regression. Fig. 8a shows plasma exposure data sampled on day 22, as well as model predictions. Parameters are detailed in Table 7.

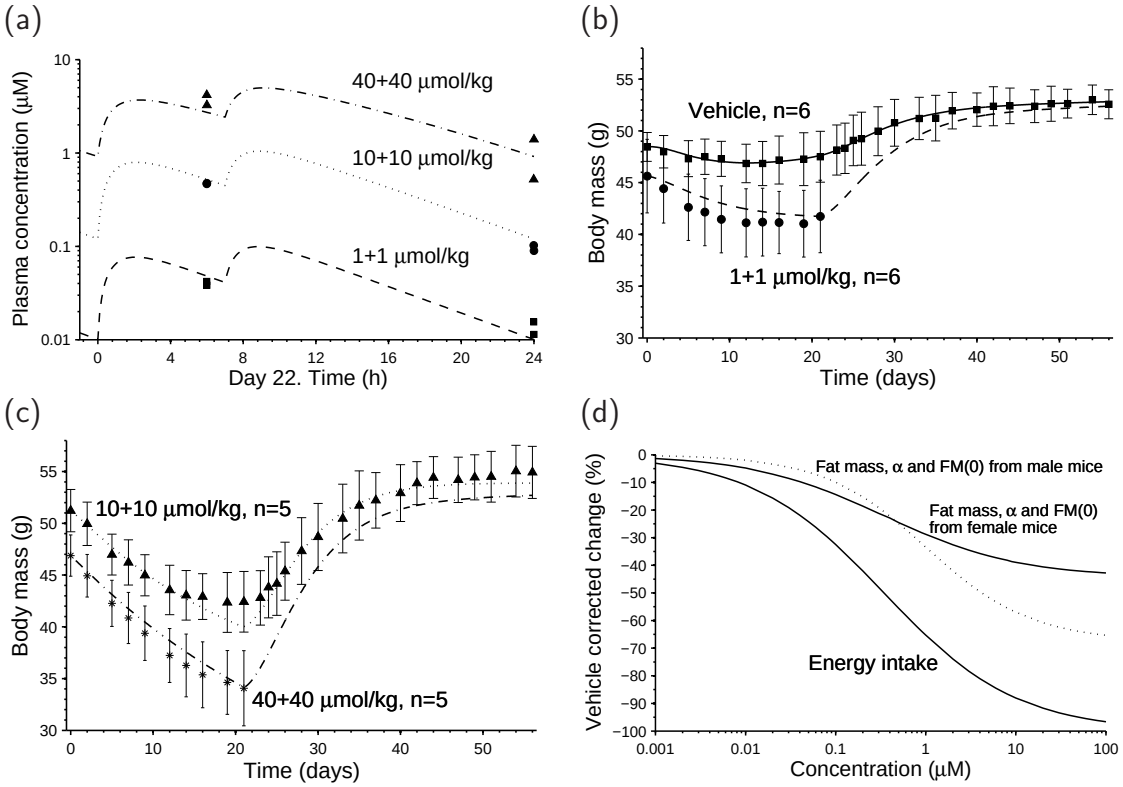

Figure 8: Modeling body mass when energy intake is not measured (Part III). ***a*** *Exposure data and PK model simulation.* ***b-c*** *Fitting the body composition model to body mass data (free parameters on physical activity and energy intake).* ***d*** *Vehicle corrected change in fat mass (after three weeks), and energy intake,* versus *steady-state concentration calculated from the inferred model for a mouse with an initial weight of 50 g. For comparison, the dotted line represents the fat mass to steady-state concentration relationship that would have been obtained if the full analysis had been executed with α and $FM(0)$ obtained from male mice*

We model EI_{veh} by Eq. 19. The compound is assumed to inhibit energy intake by Eq. 2. The resulting model is hence obtained as Eq. 20 which in turn is based upon Eq. 16 to Eq. 18. As in Part II, the body composition model is composed of the two fundamental energy equations (Eq. 7 and Eq. 8), complemented by the energy expenditure model (Eq. 11 and Eq. 12), as well as the empircial curve relating changes in fat and fat-free masses (Eq. 14). Initial conditions are obtained from Eq. 22 and Eq. 23. In the present example, prior information indicates that the effect of the compound on energy expenditure is negligible, i.e. $S(C) = 1$ (Eq. 21, Fig. 2). For the inhibitory drug action $I(C)$, the average steady-state concentration, $C_{ss,average}$, is used.

Body mass data were pooled within each group and all groups were simultaneously fitted. Observed body

Table 7: Parameter values obtained for Part III, when body mass is modeled in the absence of energy intake data. param. = parameter

Param.	Unit	Description	Value	CV%	Reference
PK					
k_a	h^{-1}	Absorption rate	1.0	fixed	Prior information
V	$L \times h^{-1} \times kg^{-1}$	Volume of distribution	10.1	19	Data in Fig. 8a
V_{max}	$\mu M \times h^{-1}$	Maximum elimination rate	1.16	35	Data in Fig. 8a
K_M	μM	Michaelis-Menten constant	7.11	33	Data in Fig. 8a
PD					
λ	$kcal \times g^{-1}$	Physical activity	0.122	37	Data in Fig. 8b–c
r_1	$kcal \times d^{-1}$	Param. in EI_{veh} (Eq. 19)	15.0	26	Data in Fig. 8b
r_2		Param. in EI_{veh} (Eq. 19)	0	fixed	Data in Fig. 8b
r_3	$kcal \times d^{-1}$	Param. in EI_{veh} (Eq. 19)	3.18	13	Data in Fig. 8b
r_4	d^{-1}	Param. in EI_{veh} (Eq. 19)	0.241	8.3	Data in Fig. 8b
r_5	$kcal \times d^{-1}$	Param. in EI_{veh} (Eq. 19)	22.4	14	Data in Fig. 8b
r_6		Param. in EI_{veh} (Eq. 19)	0.554	4.0	Data in Fig. 8b
I_{max}		Inhibitory efficacy param. in $I(C)$ (Eq. 2)	1	fixed	Data in Fig. 8b–c
IC_{50}	μM	Potency param. in $I(C)$ (Eq. 2)	0.334	2.5	Data in Fig. 8b–c
n		Sigmoidicity factor in $I(C)$ (Eq. 2)	0.592	4.0	Data in Fig. 8b–c

mass and model predictions are shown in Fig. 8b–c The final parameters are given in Table 7. Given the model, we can derive the steady-state relationship between key body composition variables, such as fat mass and energy intake, and test-compound concentration (Fig. 8d). To investigate the sensitivity of the analysis with respect to prior information on fat-mass changes, we repeated the full analysis with α and $FM(0)$ obtained from male mice. The data indicate a major shift in the relationship between fat mass and steady-state concentration (dotted line in Fig. 8d). This stresses the importance of generating data to quantify the relationship between fat and lean mass for every animal model.

Discussion

Part I. Modeling energy intake

Part I presents an example of the compound directly inhibiting energy intake rate (i.e. minimal or no time delay between compound exposure and response). If extended time delays are observed, the model can be extended using receptor binding models or turnover transduction steps.

In most energy intake studies, animals are treated with test compound for a few days. Energy intake is then continuously monitored over the treatment period to assess potential drug tolerance development or feedback mechanisms caused by an energy intake deficiency. This allows more representative and reliable predictions of the steady-state relationship between energy intake and drug concentration. In general, energy intake can be modeled over the entire drug treatment period by incorporating feedback mechanisms into the model, although the last day of recording energy intake may be the most representative for extrapolation, assuming that potential feedback has reached steady state.

In the presented modeling approach we explicitly model energy intake of vehicle animals. Based on experience, this method gives similar estimates of compound parameters as for vehicle-corrected data. The advantages of explicitly including the vehicle are more convenient data handling and improved model interpretation.

In case energy intake rate is not limited by the energy intake rate of the vehicle group, it is reasonable to extend the basic model by feedback on energy intake. The extension reflects an increased appetite in order to maintain homeostasis, and ensures that energy intake can temporarily be greater than that of control animals. Naturally, there are several plausible ways of specifying this feedback. Disease- and target-specific properties should also be taken into account.

Part II. Modeling body composition

A mathematical description of the specific in-house *in vivo* model provides a quantitative framework for reasoning about energy intake and body composition studies. In our experience, it adds value across drug discovery projects by improving study design, analysis, and interpretation of such studies. Applications include prediction of body mass over extended periods (weeks) based on food intake collected during only brief periods (days), prediction of body composition (allowing reduced sampling), and investigation of how a compound affects energy intake and energy expenditure.

A fundamental requirement of the body composition model is data for estimating the empirical function α, which specifies the relationship between changes in fat-free mass and fat mass. Our data indicate that α differs across animal models.

The body composition model is sufficiently flexible to fit data generated for energy intake and body mass. Under- or over-fitting of noisy body-mass time profiles and uncertainty in empirically based estimation of energy expenditure are major challenges when using the model. Choosing free parameters is central for the application of any of the models in this class. Some general guidance is that the sensitivity of parameters related to unobserved variables is generally high, and it is advisable to keep the physical activity parameter λ free when energy expenditure is unobserved. The identification of all energy expenditure parameters requires extensive data over a large range of perturbations, and a pragmatic approach is to fix some of the parameters, as we have done for K in the examples. Estimates of energy expenditure are still valid, but the magnitude of λ should be interpreted with caution as λ may compensate for an incorrectly chosen fixed value of K.

Part III. Modeling body mass when energy intake is not measured

Generally, observations or predictions of energy intake and energy expenditure are of major importance in order to identify a reasonable compound-induced-effect model based on the mechanism of action. Part III considers the case where no observations of energy intake or energy expenditure are available. To achieve a feasible modeling problem, we now require a relatively good understanding of energy expenditure in the animal model and also prior information of how the compound affects energy intake and energy expenditure. Therefore, we have assumed a body composition model as described in Part II.

If prior information on energy expenditure is detailed, one can also use model identification (deconvolution) methods to infer trajectories of energy intake (and not only constant energy intake rates). Since models are represented by ordinary differential equations that are non-linear in their parameters, and since input data are incomplete, deconvolution methodologies designed for sparsely observed non-linear systems are required [27].

Using either models from Part I and II together or models of Part III gives a complete integrative PKPD model from exposure to body mass. Such models enable new ways of analyzing experimental designs in the obesity field. As an example, a key question is how well a model can predict body mass change after a certain period of time given data for an initial part of that period, and when certain variables are observed. Another example is to quantify the gain in parameter precision of having a washout period in body mass studies over weeks.

Translation

A natural extension to the approaches presented here is to consider model-based translation across different species [28]. To characterize the translational capabilities of the body composition model, we compare the steady-state response of the mouse model presented in Part II with that of a human model [20] (Table 8). Two striking features emerge. First, an increase in energy expenditure (e.g. 10%) is more efficient than an equal lowering of energy intake. Second, the relative change in both body mass and fat mass is greater in human at the same relative change in either energy intake or energy expenditure. We acknowledge that the comparison assumes the same drug mechanism across species and over time. In practice, clinical data indicate that weight loss typically plateaus after roughly one year, which is much earlier than the model-predicted time to steady state. There may be several reasons for this plateau, such as functional adaptation on the level of energy intake or energy expenditure (e.g. altered target expression), compliance to treatment or exercise recommendations, and altered life situation. Unraveling those factors is challenging, but may be feasible with proper clinical designs and observations, together with model-based analyses. We see a

Table 8: Predicted steady-state effects on body mass and fat mass for drug treatments affecting energy intake and/or energy expenditure across species. Mouse predictions are based on the model presented in Part II for a female animal with an initial body mass of 50 g. Human predictions are based on the Hall (2011) model [20], assuming an obese female with a Body Mass Index (BMI) of 35 kg×m^{-2}, and with low physical activity

Drug mechanism		Body mass (% change)		Fat mass (% change)	
Energy intake	Energy expenditure	Mouse	Man	Mouse	Man
-10%	-	9	15	15	25
-5%	+5%	12	16	19	28
-	+10%	15	18	24	31

great opportunity to pursue these tracks in future research.

Identifiability

To avoid problems with parameter identifiability, it is advisable to keep parameters with strong prior information fixed. For the body composition model in particular, several parameters have a clear biophysical interpretation (such as ρ and γ), and reasonable values can be derived or estimated. Therefore, in our work we have kept them fixed, whereas other parameters (such as λ and IC_{50}) were estimated from data.

Recommendations

Based on our analyses in this paper and on experience from other data sets not included here, we give the recommendations below.

- Establish a Forbes function α experimentally for the relevant *in vivo* animal model. This is done once, and is crucial for proper interpretation of body composition predictions. Given α, regular fat mass observations can be avoided or at least conducted less frequently.
- Always include a vehicle group, consider using several dose groups, and include a washout period for improved parameter precision (see Fig. 8c for an example). Traditionally, 2–3 dose groups are used, with 5–10 animals per group. Using a model-based approach, this design can be challenged. In many cases, more information can be expected from the same number of animals using a design with more dose groups (>4) and fewer animals per group (3–4). Finally, including a group on a standard diet may improve system understanding, in particular the energy intake part of the model, and the parameter EI_{stand} in Eq. 10. It is also of interest in obesity, since a change of diet is often recommended in addition to drug therapy.
- For compounds affecting both energy intake and energy expenditure, body-mass observations should be combined with energy-intake or energy-expenditure observations, or both; for compounds affecting either energy intake or energy expenditure, it suffices to observe body mass if the body composition model is well determined.
- The models presented in this paper can be used to extrapolate body composition over time, if prior information of potential functional adaptation is available. For humans, compliance must be considered as well.
- In drug discovery, energy intake studies covering a few days can be used for initial compound ranking (Part I model). A longer study (in the order of ten days), with observations of both energy intake and body weight, can be used to determine drug action mechanisms (Part I and II model). Extended time frames (several weeks), probably with only body mass observations for cost reasons, are essential for investigating repeated dosing and functional adaptation.

Conclusions

Body composition depends on energy intake and energy expenditure, and is a key endpoint in many drug discovery and clinical studies. Three conceptual mathematical models (Parts I–III) can describe

the relationship between compound exposure, energy balance, and body composition. Key advantages of a model-based analysis include improved quantitative system understanding, improved ability to predict beyond the data ranges, and the potential to significantly improve the experimental design. It also gives the opportunity to partly replace certain analyses by model predictions.

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