Supplemental Data

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A Mathematical Model of Murine Metabolic Regulation by Leptin: Energy Balance and Defense of a Stable Body Weight

Joshua Tam, Dai Fukumura, and Rakesh K. Jain

Computational method

Model computations were performed using MatLab (The MathWorks, Natick, MA). Ordinary differential equations were solved numerically using the Dormand-Prince Runge-Kutta method. Data fitting was performed by unconstrained nonlinear optimization.

Major assumptions

The following are the major assumptions made in formulating this model:

1) We assumed that energy metabolism responds to leptin concentration, rather than other possible inputs such as the rate of change of leptin concentration. We make this assumption because most experimental reports in the literature use leptin concentration as the basis for analysis, but it should be noted that this may reflect literature bias rather than any scientific rationale for favoring one mode of leptin regulation over another.
2) This model only considers intermediate time-scales involved in body weight change (days – weeks). Events that occur on shorter time scales (seconds to hours), such as food intake, energy absorption from the gut, and leptin production and transport, are assumed to be both instantaneous and continuous. For the same reason, diurnal variations in leptin production, food intake, energy expenditure, etc., are generally disregarded. On the other hand, longer termed changes related to chronic obesity and aging are also not taken into account in the current model.
3) It is assumed that food intake control is based on food mass, rather than its energy content. This is because satiety/fullness after a meal typically occurs before the energy from the food eaten is digested and absorbed, in other words the decision to end a meal is typically reached before the energy content of that meal is known to the body.
4) This model considers only the energy content of the ingested food. Effects caused by different macronutrient compositions in the diet are ignored.
5) For this model body weight is calculated as the sum of fat mass (FM) and fat-free mass (FFM). FFM is assumed to be constant, i.e. all energy input is stored as fat, and all energy expenditure is taken from fat. This model is only intended to simulate energy homeostasis in adults with relatively stable internal organs, bone, and muscle mass.
6) Our model only deals with dietary energy content, ignoring their macronutrient composition.

Derivation of equations and parameters

Because of the well-documented effects of strain background, age, and gender on energy metabolism, we only used data from adult (6 – 24 weeks of age) male mice of the C57Bl/6J background, fed standard mouse chow, for derivation of model parameters, unless otherwise noted.

Leptin is produced and secreted by fat cells at a rate roughly linear to total fat tissue mass, and cleared mainly (>98%) by the kidney by an insaturable process consistent with glomerular filtration (Cumin et al., 1997). This relationship is described as:

$$\frac{d\left(Lep_{plasma} \times BloodVolume\right)}{dt} = FM \times R_{syn} - GFR \times RenClearance \times Lep_{plasma} \quad (1)$$

Where Lep_{plasma} is the plasma concentration of leptin. FM is fat tissue mass. R_{syn} is the leptin synthesis rate ~ 3.6 ng/100 g fat tissue/min (similar in rats and humans, assumed to be the same in mice) (Klein et al., 1996; Zeng et al., 1997). BloodVolume is the total blood volume in a mouse, which varies with body weight. In this model blood volume is estimated as $\{BloodVolume = (0.022 \times BodyWeight + 1.5)ml\}$, which is derived from the report by Yen *et al* (Yen et al., 1970) with the assumption that the relationship between body weight and blood volume is linear.

The rate of leptin removal by kidneys (*RenClearance*) is approximately 25% (Cumin et al., 1997). GFR is the glomerular filtration rate, we estimated GFR to be ~11.85ml/hr by taking the average of GFR reported in two independent studies using male C57Bl/6J mice (Dickinson et al., 2007; Qi et al., 2004). For a mouse with ~2 ml total blood volume, this gives a leptin plasma half-life of ~ 28 min. Literature values for leptin plasma half-life vary over a very wide range, from 9.4 minutes (Zeng et al., 1997) to several hours (Ahima et al., 1996).

Leptin enters the brain both by saturable receptors and by a non-saturable linear process (Banks et al., 2000; Schwartz et al., 1996). This relationship is represented as an equation taken from Banks *et al* (Banks et al., 2000):

$$Lep_{Brain} = k_1 \frac{Lep_{Plasma}}{k_2 + Lep_{Plasma}} + k_3 (Lep_{plasma}) \quad (2)$$

Banks *et al* give values of k_1 = 1.42 ng/g and k_2 = 15.6 ng/ml. From a graph in the same report showing nonspecific transport of leptin, we estimated the value for k_3 to be 0.00272 ml/g.

Leptin uptake into the CNS is not accounted for in the plasma leptin balance equation (equation 1). We believe this simplification is justifiable on the grounds that 1) plasma leptin concentration (physiological range ~ 5-40 ng/ml) is typically much higher than leptin concentration in the brain (in the 1-2 ng/g range); and 2) compared to blood volume (~2 ml in mice), brain mass (~450 mg) is relatively low, so that the amount of leptin in circulating blood dwarfs the amount of leptin in the brain.

Settling point model

In general, food intake is highest at low leptin levels, and decreases with rising leptin levels. We used a modified form of the classic Michaelis-Menten equation to describe the relationship between food intake and leptin concentration as follows:

$$FoodIntake = k_4\left(1 - \frac{Lep_{Brain}}{k_5 + Lep_{Brain}}\right) \quad (3)$$

In this equation, k_4 scales the maximum food intake value, which is obtained when leptin concentration approaches zero, therefore k_4 is equal to food intake in leptin knockout mice. Table S1 shows food intake values in leptin knockout (*ob/ob* or *db/db*) mice, as reported in several different studies. Taking the average of these values gives k_4 = 5.6 g/day.

Plasma leptin and food intake values for wild-type (WT) mice from several reports in the literature are listed in Table S2. Leptin concentration in the brain is seldom reported in the literature, and was not reported in any of the references listed in the table. We calculated brain leptin concentrations by substituting the reported plasma leptin concentrations into equation 2. Equation 3 was fitted to brain leptin and food intake data listed in Table S2 to obtain k_5 = 0.55 ng/g (Figure S 1A).

The relationship between energy expenditure and body weight/leptin levels is unclear, with seemingly contradictory reports in the literature (Table S3). Most studies showed that leptin increases energy expenditure in leptin knockout animals, or WT animals during starvation, but has little effect on energy expenditure in normally fed WT animals. Thus the effect of additional leptin seems most prominent when leptin levels are low, but when leptin levels are at normal, well-fed levels, additional leptin has little effect on energy expenditure. Again we used a modified Michaelis-Menten equation to describe energy expenditure (E_{out}) as follows:

$$E_{out} = k_6 BM\left(1 + k_7 \frac{Lep_{Brain}}{k_8 + Lep_{Brain}}\right) \quad (5)$$

In leptin knockout mice, where leptin levels are constantly zero, energy output is directly proportional to body mass (McClintock and Lifson, 1957). Energy expenditure data from various references for mice with disrupted leptin pathway are listed in Table S4. For leptin pathway knockout animals, equation 5 becomes $E_{out} = k_6 BM$. Taking the average of the data in Table S4, we obtain k_6 = 10.18 cal/g body weight/hour (Figure S 1B).

When Lep_{Brain} approaches infinity, equation 5 becomes $E_{out} = k_6 BM(1+k_7)$, thus the parameter k_8 determines the maximal asymptote for equation 5. According to Mistry et al (Mistry et al., 1997), oxygen consumption (a surrogate measurement for energy expenditure) in *ob/ob* mice was approximately 3.0 ml/g body weight/hr, while the value for WT mice was ~ 6.1 ml/g body weight/hr. Oxygen consumption for WT mice did not increase even after intracerebroventricular administration of high dose leptin, therefore it

can be assumed that the maximal asymptote has been reached. Using the two values for oxygen consumption in the equation [$E_{out} = k_6BM(1+k_7)$] gives $k_7 \sim 1$.

We then used data from WT animals (Table S5) to estimate values for k_8. Here again brain leptin concentrations were calculated by substituting the reported plasma leptin concentrations into equation 2. Equation 5 was fitted to the data listed in Table S5, giving $k_8 = 0.22$ ng/g.

The range of values used for sensitivity analysis, as well as the justification for each range, is listed in Table S6.

Set-point model

We modeled the set-point hypothesis as a feedback system regulated by proportional-integral (PI) controllers. PI controllers are a well-established class of controllers commonly used in feedback control systems, mathematically defined by the following equation (Stephanopoulos, 1984):

$$c(t) = K_c\varepsilon(t) + \frac{K_c}{\tau_1}\int_0^t \varepsilon(t)dt + c_s$$

Where $c(t)$ is the value, at time t, of the entity being regulated by the controller. K_c is the proportional gain of the controller. τ_1 is the integral time constant. $\varepsilon(t)$ is the error signal (i.e., the difference between the measured value and the desired set point) at time t. c_s is the controller's actuating signal when $\varepsilon = 0$ (also known as the "bias signal").

The PI controller was chosen because it is widely used and well-characterized, and because it fulfills the requirement of the set-point hypothesis that the controlled value eventually returns to the set-point. Although our analysis was based on simulations using PI controllers, the conclusions are applicable to any control system that is able to return its output to a state of zero error. The simplest controller (proportional controller) was not used because proportional controllers suffer from offset, such that there is always a discrepancy between the response to either a new set point or to a persistent change in load, thus violating the central tenet of the set-point hypothesis.

We assume that whole brain leptin level is the measured signal. For the set-point model, food intake is defined as:

$$FoodIntake(t) = a_1\left(Lep_{Brain}(t) - SetPt\right) + a_2\int_o^t \left(Lep_{Brain}(t) - SetPt\right)dt + c_1 \qquad (8)$$

Where SetPt is the brain leptin set-point. For consistency and ease of comparison, we used the steady-state brain leptin level obtained in our previous simulation (0.34 ng/g) as the set-point. c_1 is the amount of food intake when Lep_{Brain} equals to the set-point. Again this is set to be the same as the steady-state value of the previous model (3.56 g/day).

Similarly, energy output (per unit body weight) can be defined as:

$$E_{Out}(t) = BM \times \left(a_3\left(Lep_{Brain}(t) - SetPt\right) + a_4\int_o^t \left(Lep_{Brain}(t) - SetPt\right)dt + c_2 \right) \qquad (9)$$

Where c_2 = energy output when Lep_{Brain} equals to the set-point, from the steady-state solution of the previous model, c_2 = 16.34 cal/g/hr.

Because of the integral terms in equations 8 and 9, the values for parameters $a_1 – a_4$ cannot simply be fitted to experimental data as we did for the parameters in the settling point model. Instead, here we have set physiological upper and lower bounds for food intake and energy expenditure values, and arbitrary chose values for $a_1 – a_4$ such that the system is stable and does not oscillate. While the values for $a_1 – a_4$ affect the dynamic behavior of the system, they affect neither the steady state values nor the conclusions drawn from this model. Food intake is set to a maximum of 5.6g/day (the average amount of food intake by leptin pathway knockout mice, Table S1), and a minimum of 0. Energy expenditure is set to a minimum of 10.18cal/g/hr (energy output in leptin pathway knockout mice, Table S4), and a maximum of twice this value (20.36 cal/g/hr, see derivation of equation 5 above for justification).

There are two key differences between the "settling point" and "set-point" systems, as currently defined: 1) while the steady-state solution in the settling point system is a product of the functions governing food intake and energy output, and the various parameters contained in these functions, the set-point system is driven by the attempt to maintain leptin levels at an explicitly defined level; and 2) the set-point system is able to return the system to the set-point despite persistent change in input, which is impossible for a settling point type system.

Our justifications for designing a set-point model that is able to completely eliminate even small errors are as follows:

1. The prevailing conceptualization of the set-point model depicts a system that does return to the set-point. This view was shaped mainly by the observation that people who intentionally try to change their body weight, whether they are trying to lose weight (e.g. obese persons going on diets) or trying to gain weight (e.g. actors trying to gain weight for movie parts), are usually able to do so for a short time, but eventually they return to their original body weight. This led to the conclusion that one's body weight can be transiently perturbed, but eventually the body weight does return to the set-point.

2. There are experimental examples in some animals of the type of precise control that is only possible in a set-point system that is able to completely eliminate even small errors. For example, rats (Adolph, 1947) and mice (Dalton, 1965) appear to be able to maintain their body weights very well against dilutions in dietary caloric content. In both cases the animals were able to maintain virtually constant body weights despite up to 50% diet dilution. In mice, significant weight loss did not occur until diet dilution was so severe (70%) that the animals began to fall ill and even die (Dalton, 1965).

   Interestingly, not all animals are able to maintain their body weights when challenged with dietary dilution. For example, cats loss weight roughly linearly

with the extent of dilution in their diets (Hirsch et al., 1978). These animals would not be consistent with a model that completely returns to the set-point.

Humans have shown a variety of responses towards dietary dilution. Some people are able to adjust their meal sizes to maintain stable body weights despite dietary dilutions, while others are not (Spiegel, 1973). It is possible that variations in the relative dominance of the set-point component versus the settling point component could account for differences between individuals.

Leptin haploinsufficiency

In the text we simulated leptin haploinsufficiency by decreasing leptin synthesis rate by 50%, and compared the change in percentage body fat predicted by the model to published experimental results (Chung et al., 1998). It should be noted that the animals used by Chung *et al* have a different baseline steady-state (2.7g body fat for widetype mice) than the studies from which we derived our modeling parameters (~6g body fat for C57 widetypes. Reed *et al*, Physiology & Behavior, 2007), so more direct comparisons (e.g. in absolute fat mass) is unfortunately not possible without more information on the experimental conditions (food intake, energy expenditure, etc. for widetype and heterozygotes).

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Supplementary tables and legends

Table S1. Literature values for food intake in leptin pathway knock-out (*ob/ob* and *db/db*) mice.

Food intake (g/mouse/day)	Reference
4.64	(Saito and Bray, 1984)
5.5	(Szczypka et al., 2000)
4.5	(McClintock and Lifson, 1957)
5.16	(Hwa et al., 1997)
8.05	(Hwa et al., 1997)
6	(Qiu et al., 2001)

Table S2. Literature values for leptin levels and food intake in wild-type, young adult C57Bl/6J males.

Plasma leptin (ng/ml)	Brain Leptin (ng/g)*	Food intake (g/mouse/day)	Reference
2.2	0.18	3.6	(Jacobson, 2002)[#]
4	0.3	3.7	(Jacobson, 2002)[#]
2.25[+]	0.19	4.5	(Beattie et al., 1998)
2.5	0.2	4.3	(Raposinho et al., 2001)

* Leptin concentration in the brain was estimated from plasma leptin, using equation 2

[#] This reference also reported data on mice that were 11-12 months old. That data was not used because the mice were too old.

[+] Average of leptin levels given at two different ages (~2ng/ml at 7 weeks and ~2.5ng/ml at 22-39 weeks).

Table S3. Effect of leptin on energy expenditure, according to reports in the literature

Conclusions	Reference
Energy expenditure is roughly linear to body weight in genetically obese mice, and higher than lean littermates	(McClintock and Lifson, 1957)
Leptin increases energy consumption in *ob/ob* mice, and in food deprived lean mice	(Mistry et al., 1997)
Leptin increases energy expenditure of food-restricted lean mice, but when food is abundant only changes food intake	(Doring et al., 1998)
Leptin infusion reduces fat mass in excess of that caused only by reduction in food intake (presumably by increasing energy expenditure)	(Levin et al., 1996)
Energy expenditure per unit body weight is increased after 3 weeks of leptin administration in *ob/ob* mice, but no effect in lean mice	(Pelleymounter et al., 1995)
Energy expenditure unchanged by central leptin infusion	(Halaas et al., 1997)
Leptin increases energy expenditure (normalized to body size) in *ob/ob* mice, but not in lean ob+/- mice	(Hwa et al., 1997)
No relationship between energy expenditure and body mass (within narrow range: 20-28g)	(Johnston et al., 2007)

Table S4. Energy expenditure data for leptin pathway knockout (*ob/ob* and *db/db*) mice.

Genotype	Mean body weight (g)	Energy expenditure (kcal/day)	Energy expenditure /body weight (cal/g/day)	Reference
Ob/ob	28.2*	6.91	244.8	(McClintock and Lifson, 1957)
Ob/ob	35.4*	8.69	244.8	(McClintock and Lifson, 1957)
Ob/ob	53.5*	15.1	283.2	(McClintock and Lifson, 1958)
Ob/ob	37	7.7^{+}	206.4	(Hwa et al., 1997)
Db/db	37.8	9.25^{+}	244.8	(Hwa et al., 1997)

* When body weight was reported as a range, mean body weight is estimated by averaging the body weights at the two ends of the range. E.g. For the first entry Ob mice weigh 23.7g at week 6 and 32.7g at week 9, therefore the average body weight for weeks 6-9 = (23.7+32.7)/2 = 28.2g

$^{+}$ Energy expenditure was calculated from indirect calorimetry data using this equation (Simonson and DeFronzo, 1990): $E = (3.91 + 1.10\ RQ)\ V_{O2}$, where E = energy expenditure (in kcal/min), RQ = respiratory quotient, V_{O2} = oxygen consumption (in L/min). RQ ~ 0.98 was given in the reference.

Table S5. Energy expenditure and corresponding leptin data for wild-type mice.

Body mass (g)	Energy* expenditure (kcal/day)	Plasma leptin (ng/ml)	Brain leptin# (ng/ml)	Reference
28	11.4	4.4	0.32	(Kennedy et al., 2007)
27.5	9.84+	4.31	0.28	(Patel et al., 2006)
25	10.1	5	0.36	(Chen et al., 2000)**

* Estimated from graphs given in the corresponding references

Estimated values using equation 2 .

+ Calculated from indirect calorimetry data using the equation $E = (3.91 + 1.10\ RQ)\ V_{O2}$. RQ assumed to be 0.85.

** Data from this reference were from mice of mixed 129Sv-C57Bl/6J strain background

Table S6. Range of values used for sensitivity analysis.

Parameter	Range	Justification
k_1	1.25 – 2.7	Range given by Banks et al (Banks et al., 2000) for different regions of the brain
k_2	15.6 – 31	
k_3	±1/3	*
k_4	4.5 – 8.05	Range of literature data, as listed in Table **S1**
k_5	±1/3	*
k_6	206.4 – 283.2	Range of literature data, as listed in Table S4
k_7	±1/3	*
k_8	±1/3	*
R_{syn}	±1/3	*
GFR	252 – 316.8	Values reported in (Dickinson et al., 2007; Qi et al., 2004)
RenClearance	n/a	+
ρ_{food}	3.2 – 5.25	Low end = chow diet, high end = 60% high fat diet

* Literature values not available for these parameters, the range is estimated to be ±1/3 of the corresponding value as listed in Table 1 in the main text. This estimation is based on the range of the other parameters k_1,k_2,k_4, and k_6, where there is approximately 2x difference between the minimum and maximum values.

+ In this model changes in RenClearance is equivalent to changes in GFR (see equation 1), therefore sensitivity analysis is not repeated for RenClearance.

Supplementary Figure Legends

Figure S 1

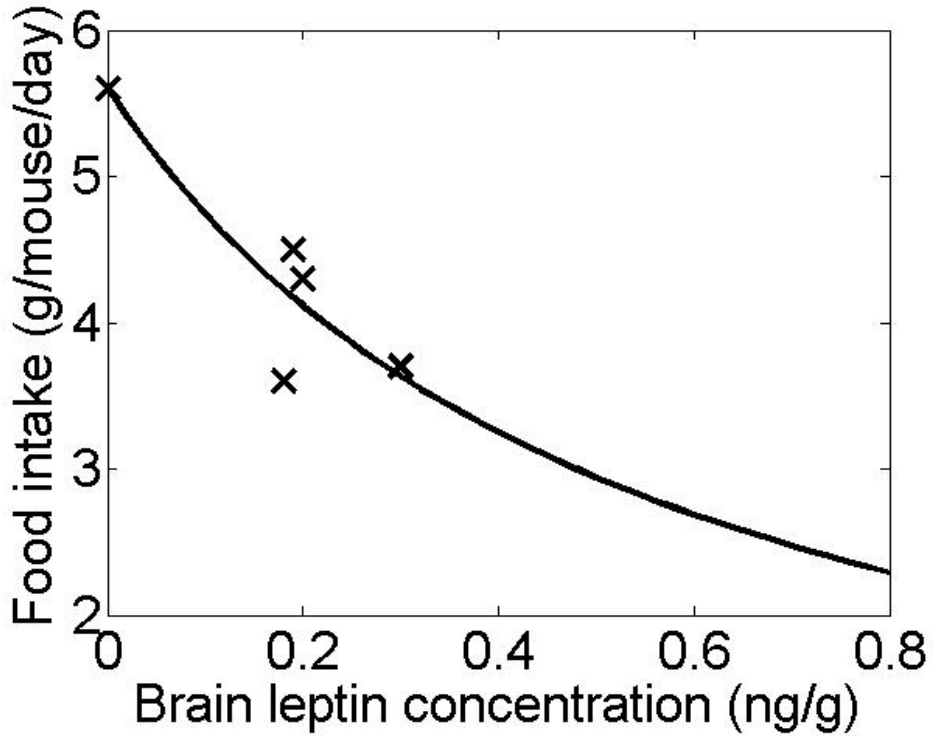
Leptin dose-response curves for (A) food intake (g/mouse/day), and (B) energy expenditure (cal/g body weight/hour) for the settling point model (equations 3 and 5). Solid curves represent calculated food intake and energy expenditure according to their corresponding equations, as described in the text. Crosses represent experimental data (Tables S2 and S5) used to obtain the corresponding model parameters.

Figure S 2

Sensitivity of the settling point model towards variations in individual parameters ($k_1 - k_8$, Rsyn, GFR, and ρ_{food}). The specific model parameter being varied is stated in the x-axis of each graph. Parameters are varied from across its physiological range (Table S6). Note that "physiological range" represents the variability of each parameter in healthy animals under standard experimental conditions. Pathological states such as low GFR due to renal failure, or special manipulations such as dietary dilution or exposure to taxing metabolic environments (e.g. prolonged cold temperature) are not represented in this analysis. In this model, changes in RenClearance are equivalent to changes in GFR (see equation 1), therefore sensitivity analysis was not repeated for RenClearance.

Figure S 1

A:


Food intake (g/mouse/day)
6
5
4
3
2
0
0.2
0.4
0.6
0.8
Brain leptin concentration (ng/g)

B:

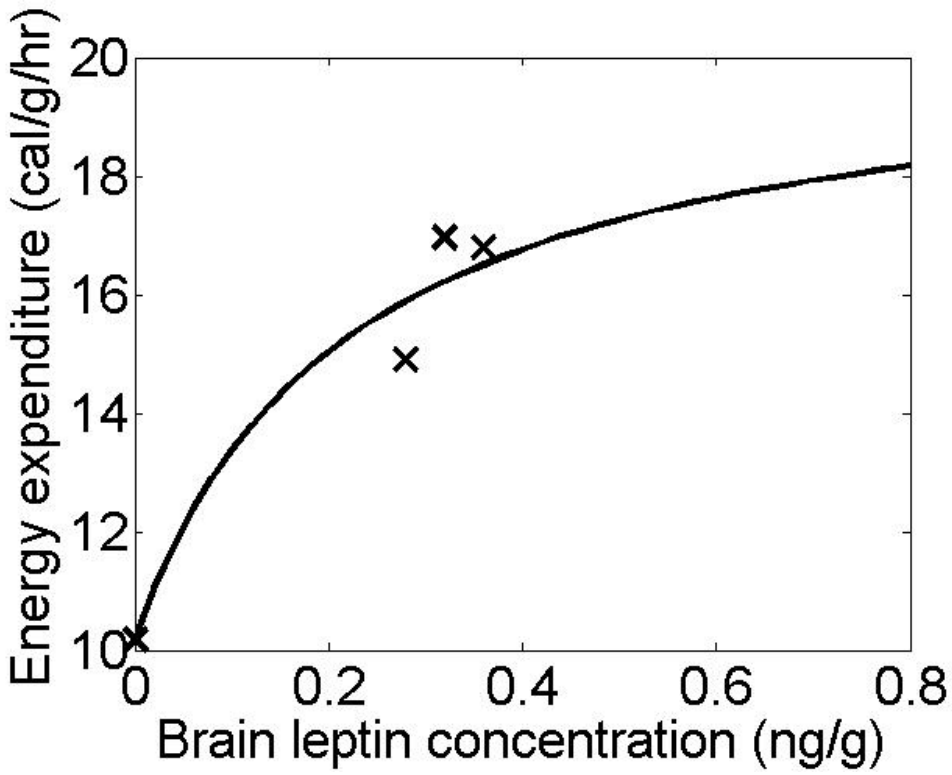
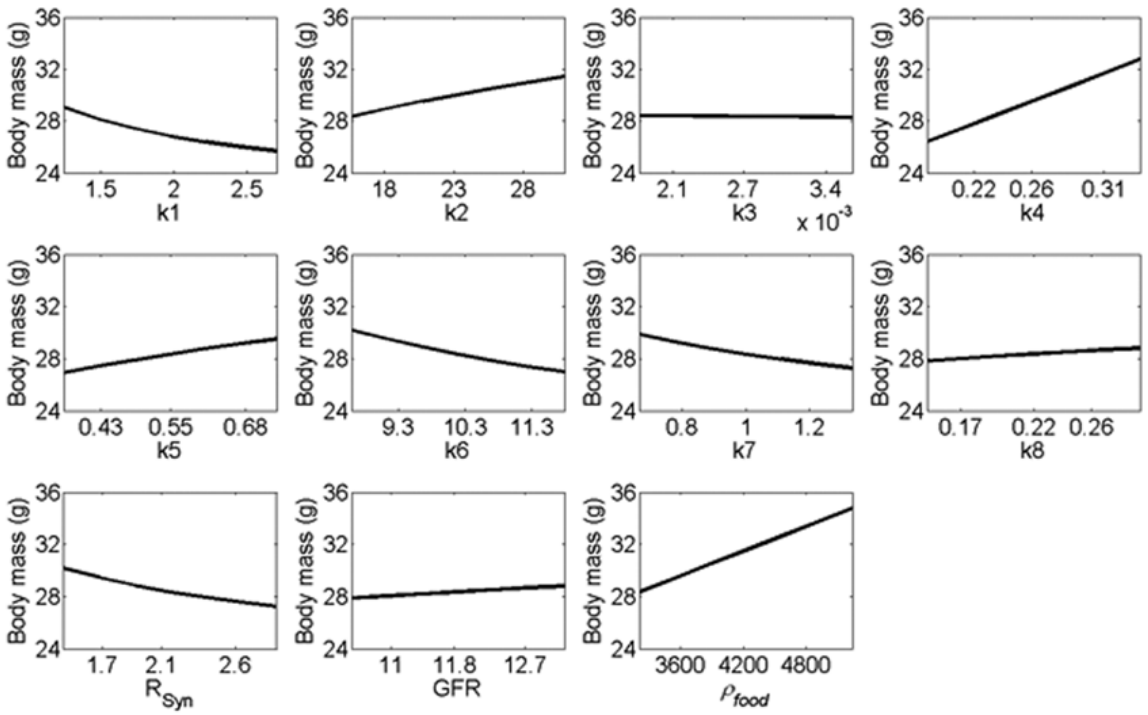

Energy expenditure (cal/g/hr)
20
18
16
14
12
10
0
0.2
0.4
0.6
0.8
Brain leptin concentration (ng/g)

Figure S 2


Body mass (g)
36
32
28
24
1.5
2
2.5
k1
18
23
28
k2
2.1
2.7
3.4
k3
x 10^-3
0.22
0.26
0.31
k4
0.43
0.55
0.68
k5
9.3
10.3
11.3
k6
0.8
1
1.2
k7
0.17
0.22
0.26
k8
1.7
2.1
2.6
R_Syn
11
11.8
12.7
GFR
3600
4200
4800
ρ_food