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# Mathematical modeling of the hormonal regulation of food intake and body weight

## Applications to caloric restriction and leptin resistance

**Thèse de doctorat**

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# Résumé


## Modélisation mathématique de la régulation hormonale de la prise alimentaire et de la prise de poids Applications à la restriction calorique et la résistance à la leptine

Réguler la prise alimentaire et la dépense énergétique permet en général de limiter d'importants changements de poids corporel. Hormones (leptine, ghréline, insuline) et nutriments sont impliqués dans ces régulations. La résistance à la leptine, souvent associée à l'obésité, limite la régulation de la prise alimentaire. La modélisation mathématique de la dynamique du poids contribue en particulier à une meilleure compréhension des mécanismes de régulation (notamment chez l'humain). Or les régulations hormonales sont largement ignorées dans les modèles existants.

Dans cette thèse, nous considérons un modèle de régulation hormonale du poids appliqué aux rats, composé d'équations différentielles non-linéaires. Il décrit la dynamique de la prise alimentaire, du poids et de la dépense énergétique, régulés par la leptine, la ghréline et le glucose. Il reproduit et prédit l'évolution du poids et de la prise alimentaire chez des rats soumis à différents régimes hypocaloriques, et met en évidence l'adaptation de la dépense énergétique. Nous introduisons ensuite le premier modèle décrivant le développement de la résistance à la leptine, prenant en compte la régulation de la prise alimentaire par la leptine et ses récepteurs. Nous montrons que des perturbations de la prise alimentaire, ou de la concentration en leptine, peuvent rendre un individu sain résistant à la leptine et obèse. Enfin, nous présentons une simplification réaliste de la dynamique du poids dans ces modèles, permettant de construire un nouveau modèle combinant les deux modèles précédents.



**Mots-clés :** Modélisation mathématique, Équations différentielles ordinaires, Modèles à retards, Régulation du poids corporel, Leptine, Résistance à la leptine

# Abstract


## Mathematical modeling of the hormonal regulation of food intake and body weight
## Applications to caloric restriction and leptin resistance

The regulation of food intake and energy expenditure usually limits important loss or gain of body weight. Hormones (leptin, ghrelin, insulin) and nutrients (glucose, triglycerides) are among the main regulators of food intake. Leptin is also involved in leptin resistance, often associated with obesity and characterized by a reduced efficacy to regulate food intake. Mathematical models describing the dynamics of body weight have been used to assist clinical weight loss interventions or to study an experimentally inaccessible phenomenon, such as starvation experiments in humans. Modeling of the effect of hormones on body weight has however been largely ignored.

In this thesis, we first consider a model of body weight regulation by hormones in rats, made of nonlinear differential equations. It describes the dynamics of food intake, body weight and energy expenditure, regulated by leptin, ghrelin and glucose. It is able to reproduce and predict the evolution of body weight and food intake in rats submitted to different patterns of caloric restriction, showing the importance of the adaptation of energy expenditure. Second, we introduce the first model of leptin resistance development, based on the regulation of food intake by leptin and leptin receptors. We show that healthy individuals may become leptin resistant and obese due to perturbations in food intake or leptin concentration. Finally, modifications of these models are presented, characterized by simplified yet realistic body weight dynamics. The models prove able to fit the previous, as well as new sets of experimental data and allow to build a complete model combining both previous models regulatory mechanisms.



**Keywords:** Mathematical modeling, Ordinary differential equations, Body weight regulation, Delay differential equations, Leptin, Leptin resistance

# Résumé étendu

## Modélisation mathématique de la régulation hormonale de la prise alimentaire et de la prise de poids Applications à la restriction calorique et la résistance à la leptine

La modélisation mathématique appliquée à la description de la dynamique du poids corporel est utilisée pour aider des interventions cliniques, par exemple pour estimer la perte de poids en fonction de différents régimes, ou pour permettre d'étudier des phénomènes pour lesquels des expériences sont impossibles, notamment la privation totale de nourriture chez l'humain, et ainsi mieux comprendre les mécanismes en jeu dans la régulation de cette dynamique.

La régulation du poids corporel est un phénomène complexe, dépendant de nombreux facteurs interconnectés. Les variations de poids sont dues à des déséquilibres de la balance énergétique, qui correspond à la différence entre l'énergie consommée et l'énergie dépensée, qui sont toutes deux régulées. L'énergie consommée dépend de la quantité et de la composition énergétique de la nourriture. La dépense énergétique est composée de différents éléments qui permettent au corps de fonctionner (métabolisme de base), de maintenir sa température (thermogenèse adaptative) ou de se déplacer (activité physique). L'apport et la dépense énergétiques sont régulés au niveau de l'hypothalamus par des hormones, en particulier la ghreline et la leptine, des nutriments, mais aussi par des signaux provenant du système digestif. La ghreline est produite essentiellement par l'estomac et sa production est inhibée lors de la consommation de nourriture, en fonction de la quantité et de la composition des aliments. Elle agit à court terme et entraine une augmentation de la prise alimentaire. La leptine est produite par le tissu adipeux et indique la quan-

tité d'énergie stockée sous forme de triglycérides. Elle induit une diminution de la prise alimentaire via l'activation de récepteurs spécifiques dans l'hypothalamus. La résistance à la leptine, souvent associée à l'obésité, induit une diminution de l'action de la leptine. Cette résistance a de multiples causes dont une diminution du transport de la leptine jusqu'à l'hypothalamus et/ou une diminution de l'activation des récepteurs. La leptine a été identifiée comme étant un régulateur de ses récepteurs, ce qui fait qu'elle est potentiellement impliquée dans le développement de la résistance. Malheureusement, les régulations hormonales sont largement ignorées dans les nombreux modèles mathématiques existants, qui se concentrent plutôt sur la dynamique des nutriments ou sur une description détaillée de la balance énergétique.

Dans cette thèse, nous présentons tout d'abord un modèle de la régulation du poids corporel et de la prise alimentaire chez le rat, prenant en compte des régulations par la leptine, la ghreline et le glucose sanguin, une adaptation de la dépense énergétique, la composition du corps (divisé en masse grasse et masse maigre) et la nourriture disponible. Ce modèle est composé d'équations différentielles ordinaires et à retard, et basé sur des travaux existants. Des expériences de restriction calorique chez les rats ont été menées spécifiquement pour tester la pertinence du modèle. Lors de ces expériences, la quantité de nourriture disponible pour chaque rat a été réduite et répartie différemment durant les 8 semaines de l'expérience : un groupe a reçu une nourriture constante chaque jour, un groupe une nourriture constante par période d'une semaine et le dernier groupe a reçu très peu de nourriture pendant 4 semaines et beaucoup durant le reste de l'expérience. Après 8 semaines, des différences significatives de poids sont observées entre les groupes mais ne peuvent pas être expliquées uniquement par la quantité de nourriture consommée, en raison d'une adaptation de la dépense énergétique tout au long de l'expérience pour limiter la perte de poids. Le modèle nous permet de reproduire et de prédire les données observées de prise alimentaire et d'évolution du poids, en particulier il est possible de prédire que de la nourriture n'est pas consommée malgré le régime hypocalorique. La description de la dépense énergétique comme dépendant d'une mémoire de la nourriture consommée (estimée à 8 jours) est essentielle à la reproduction des données expérimentales et permet d'expliquer les forts gains de poids après une période de restriction intense, étant donné que la dépense énergétique a été diminuée pour équilibrer la balance énergétique et donc limiter la perte de poids.

Ce modèle sert ensuite de base pour construire un modèle de régulation de la prise alimentaire et du poids corporel par la leptine et ses récepteurs, afin d'étudier le développement de la résistance à la leptine. En accord avec les connaissances biologiques actuelles, le

modèle est basé sur les hypothèses suivantes : la prise alimentaire est inhibée par l'activation des récepteurs par la leptine et la leptine régule la production et la dégradation de ses récepteurs, de façon à ce qu'un faible nombre de récepteurs soit associé à une importante concentration de leptine. Ce système possède un ou deux équilibres positifs stables, tels que l'un des équilibres corresponde à un état sain (avec peu de masse grasse) et l'autre à un état résistant à la leptine et obèse (avec beaucoup de masse grasse, mais peu de récepteurs). Nous montrons que des variations des paramètres peuvent entraîner un changement de l'état sain vers l'état résistant à la leptine et obèse. En particulier, une augmentation de la stimulation de la prise alimentaire peut induire, en fonction de la condition initiale et de l'amplitude de la variation, un développement de résistance à la leptine caractérisé par une forte augmentation du poids. La même variation dans le sens opposé ne permettra pas forcément un retour à la condition originale à cause d'un cycle d'hystérèse présent dans le modèle. De même des oscillations de ce paramètre peuvent induire des oscillations du poids autour de l'état sain, de l'état obèse ou entre les deux, en fonction de l'amplitude et de la fréquence des variations. Les prédictions de ce modèle sont également testées sur des données expérimentales de développement de résistance à la leptine chez des rats soumis à une infusion de leptine dans le cerveau. Notre modèle est capable de reproduire la prise alimentaire et les variations de poids observées qui sont caractéristiques du développement de la résistance : au début de l'injection, la prise alimentaire diminue fortement, suivie par le poids, avant de réaugmenter progressivement jusqu'à sa valeur initiale quelques jours plus tard malgré la forte concentration de leptine. Le modèle prédit une forte diminution du nombre de récepteurs due à la forte concentration de leptine, expliquant la perte de régulation de la prise alimentaire.

Finalement, nous proposons une simplification réaliste de la dynamique du poids corporel, consistant à réunir les équations décrivant la masse grasse et la masse maigre en une seule équation, ce qui limite le nombre de paramètres, facilite l'analyse et permet d'éviter des comportements irréalistes présents dans les modèles précédents, tel qu'une perte de positivité des solutions. Cette simplification inclut une description de la masse grasse en fonction de la masse totale, basée sur des données expérimentales. Nous testons la pertinence de cette simplification en incluant la nouvelle équation dans les modèles précédents et montrons que les dynamiques de prise alimentaire et de poids sont conservées. Les prédictions des modèles simplifiés sont aussi bonnes voire meilleures que les prédictions originelles malgré la réduction du nombre de paramètres. Nous pouvons donc proposer un nouveau modèle qui réunit les deux modèles précédents et permet de modéliser la dynamique du poids et de la prise alimentaire par les hormones leptine et ghreline, prenant en compte l'adaptation de la dépense énergétique et permettant d'étudier le développement

de la résistance à la leptine.

# Contents

**Résumé étendu** **13**

**List of Figures** **21**

**List of Tables** **25**

**Glossary** **27**

**I Introduction: body weight and food intake dynamics** **29**

I.1 Regulation of food intake and body weight in mammals . . . . . 30

I.1.1 Regulation of food intake and energy expenditure . . . . . 34

I.1.2 Hormonal regulators of food intake: ghrelin, insulin and gut hormones . . . . . 39

I.1.3 Leptin and leptin resistance . . . . . 41

I.1.3.1 Leptin . . . . . 41

I.1.3.2 Leptin receptors . . . . . 44

I.1.3.3 Leptin resistance and pathway to obesity . . . . . 47

I.2 Mathematical models of body weight dynamics . . . . . 52

I.2.1 Macroscopic models . . . . . 53

I.2.2 Modeling body composition dynamics . . . . . 55

I.2.2.1 Forbes model . . . . . 57

I.2.2.2 Models of energy balance . . . . . 59

I.2.2.3 Macronutrients dynamics . . . . . 64

I.2.3 Hormonal regulation of body weight . . . . . 68

I.3 Thesis work . . . . . 71

**II Jacquier et al. 2014** **75**

II.1 Introduction . . . . . 78

II.2 Materials and Methods . . . . . 80

II.2.1 Experimental procedures . . . . . 80

II.2.1.1 Animal care . . . . . 80

II.2.1.2 Sacrifice, blood and tissue collection . . . . . 83
II.2.1.3 Biochemical analysis . . . . . 83
II.2.2 Mathematical model . . . . . 84
II.2.3 Adaptation of energy expenditure . . . . . 87
II.2.4 Parameter estimation . . . . . 88
II.2.5 Predictions . . . . . 91
II.2.6 Statistical analysis . . . . . 91
II.3 Results . . . . . 92
II.3.1 Food availability modifies body weight dynamics . . . . . 92
II.3.2 Mathematical model of food intake and body weight evolution . . 94
II.3.3 A metabolic memory is necessary to explain the observed data . . 96
II.3.4 Hypothesis to explain body weight differences . . . . . 98
II.4 Discussion . . . . . 99

**III Jacquier et al. 2015** **103**
III.1 Introduction . . . . . 105
III.2 Methods . . . . . 108
III.2.1 Mathematical model . . . . . 108
III.2.2 Varying food consumption . . . . . 114
III.3 Results . . . . . 117
III.3.1 Existence of equilibria and stability analysis . . . . . 117
III.3.2 Constant leptin infusion can lead to leptin resistance . . . . . 121
III.3.3 Varying the stimulation rate of food intake can induce leptin resistance and obesity . . . . . 124
III.3.3.1 Increasing then decreasing food intake stimulation rate . 125
III.3.3.2 Oscillating food intake stimulation . . . . . 128
III.4 Discussion . . . . . 130

**IV A new and simplified model of body weight dynamics** **133**
IV.1 Introduction . . . . . 133
IV.2 New model of body weight dynamics . . . . . 134
IV.2.1 Description . . . . . 134
IV.2.2 Estimation of fat mass from total body weight . . . . . 136
IV.3 Hormonal regulation of body weight . . . . . 138
IV.3.1 Model . . . . . 139
IV.3.2 Analysis . . . . . 140
IV.3.3 Comparison to experimental data . . . . . 142
IV.3.3.1 Experimental data from [Jacquier et al., 2014] . . . . . 143

IV.3.3.2 Long-term dynamics . . . . . 145
IV.4 Leptin resistance . . . . . 147
IV.4.1 Model . . . . . 148
IV.4.2 Analysis . . . . . 148
IV.4.3 Comparison to experimental data . . . . . 152
IV.5 Full model of body weight dynamics . . . . . 155

**V Discussion and perspectives** **159**

**A Appendix: Analysis of a simplified system** **165**
A.1 Model formulation . . . . . 165
A.2 Model analysis . . . . . 167
A.3 Rescaling of system (A.2) . . . . . 170

**Bibliography** **171**

# List of Figures


I.1 Evolution of the prevalence of overweight and obesity from 1980 to 2013 . 32
I.2 Schematic representation of food intake regulation . . . . . 38
I.3 Representation of leptin actions . . . . . 43
I.4 Leptin receptor isoforms . . . . . 45
I.5 Leptin signaling pathways . . . . . 46
I.6 Regulation of food intake and body weight for normal and leptin resistant cases . . . . . 50
I.7 A model of obesity based on a defect of leptin transporter through the blood brain barrier. . . . . 50
I.8 Representation and results of [Horgan, 2011] model . . . . . 54
I.9 Schematic representation of [Kozusko, 2001] and [Goldbeter, 2006] models 55
I.10 Predicted evolution of fat-free mass as a function of fat mass . . . . . 57
I.11 Illustration of predicted change in fat-free mass from [Hall, 2007] . . . . . 58
I.12 Energy change, depending on energy intake and energy expenditure . . . 59
I.13 Evolution of fat mass, fat-free mass and ketone bodies during starvation . 61
I.14 Predicted temporal evolution of body weight from [Thomas et al., 2011] . 63
I.15 Schematic representation of [Hall, 2006] model . . . . . 64
I.16 Comparison between predicted and observed evolutions of body weight and macronutrients oxidation rates (from [Hall, 2010b]) . . . . . 67
I.17 Representation of a model of leptin regulation in mice [Tam et al., 2009] . 69

II.1 Daily available food per rat in each group. . . . . 82
II.2 Body weight and food intake evolution. . . . . 82
II.3 Schematic representation of the model. . . . . 85
II.4 Final experimental body weight and food intake. . . . . 93
II.5 Simulated evolution of body weight compared to experimental data. . . . 95
II.6 Evolution of cumulated food intake predicted by the model compared to experimental data. . . . . 96
II.7 Predicted body weight with a constant rate of energy expenditure $R$ compared to experimental results. . . . . 97

II.8 Predicted rate of energy expenditure variations in the cases H0, H1 and H4. . . . . 98
II.9 Predicted body weight for a 16 weeks experiment with different combinations of food availability patterns. . . . . 99

III.1 Schematic representation of the leptin-mediated regulation of food intake and the activation of leptin receptors. . . . . 109
III.2 State variable flow diagram of System (III.10). . . . . 113
III.3 Functions used to temporally modify the stimulation rate of food intake. . 116
III.4 Equilibria and bifurcation diagrams for parameters $\gamma_{FI}$ and $\lambda_{R2}$. . . . . 119
III.5 Relative values of leptin, leptin receptors and food intake during leptin injection. . . . . 122
III.6 Simulation results compared to [Pal and Sahu, 2003] experimental data. . 123
III.7 Evolution of the value of $FM$, $FM^*$, $L$ and $R$ for an increasing then decreasing sigmoid-like function $\gamma_{FI}$. . . . . 126
III.8 Final value of the fat mass $FM$ after an increase of the food intake stimulation $\gamma_{FI}$, followed by a decrease. . . . . 127
III.9 Evolution of fat mass $FM$ when the stimulation rate of food intake $\gamma_{FI}$ oscillates. . . . . 128

IV.1 Example of a simulation with all parameter values and initial conditions positive but with fat mass and leptin becoming negative . . . . . 135
IV.2 Evolution of fat mass as a function of body weight in Wistar rats. . . . . 137
IV.3 Variable flow diagram corresponding to System (IV.4) . . . . . 139
IV.4 Predictions of Systems (IV.4) and (II.1)–(II.8) for body weight and food intake compared to experimental data on group H0. . . . . 144
IV.5 Predictions of Systems (IV.4) and (II.1)–(II.8) for body weight and food intake compared to experimental data on group H1. . . . . 144
IV.6 Predictions of Systems (IV.4) and (II.1)–(II.8) for body weight and food intake compared to experimental data on group H4. . . . . 145
IV.7 Body weight evolution predicted by System (IV.4) and System (II.1)–(II.8) compared to experimental body weight for 16 weeks on the group with constant food availability. . . . . 146
IV.8 Body weight evolution predicted by System (IV.4) and System (II.1)–(II.8) compared to experimental body weight for 16 weeks on the group with alternations between low and high food availability. . . . . 147
IV.9 Variable flow diagram for the model described by System (IV.7), accounting for leptin resistance . . . . . 148

IV.10 Bifurcation diagram for System (IV.8) for parameter $\gamma_F$. . . . . 151
IV.11 Bifurcation diagram for System (IV.7) for parameter $\gamma_F$. . . . . 151
IV.12 Comparison between experimental data and models predictions in the case of continuous leptin injection, for a minimization performed for food intake. . . . . 152
IV.13 Comparison between experimental data and models predictions in the case of continuous leptin injection, for a minimization performed for food intake and body weight. . . . . 153
IV.14 Variable flow diagram of System (IV.16) . . . . . 156

A.1 Graphs of the functions $f_1$ and $f_2$ defined in (A.5) and (A.6) for three different values of $\gamma_{FM}$. . . . . 168

# List of Tables

I.1 Relationship between Body Mass Index and body weight status . . . . . 31
I.2 Summary of the characteristics and functions of hormones involved in the regulation of body weight . . . . . 39

II.1 Biometric data. . . . . 81
II.2 Plasma hormones and glucose assays. . . . . 84
II.3 Model variables . . . . . 84
II.4 Model parameters. . . . . 89
II.5 Approximate bayesian computation of parameters. . . . . 91
II.6 Akaike Information Criteria. . . . . 97

III.1 Model Variables . . . . . 109
III.2 Parameter units and default values used in the simulations. . . . . 110
III.3 Parameter values used for leptin injection simulations. . . . . 124

IV.1 Parameter values estimated for System (IV.4), on groups H1 and AL . . . 143
IV.2 Comparison of the Akaike information criteria (AIC) obtained with System (II.1)–(II.8) and System (IV.4) for the data from [Jacquier et al., 2014]. . . 145
IV.3 Comparison of the Residual Sum of Squares obtained with System (II.1)–(II.8) and System (IV.4) for the data on 16 weeks of caloric restriction . . 146
IV.4 Estimated parameter values for the new model with leptin resistance. . . . 153
IV.5 Residual sum of squares and Akaike information criterion for the System (III.10) and System (IV.7) with leptin resistance. . . . . 154

# Glossary

AIC Akaike Information Criterion, page 83

ATP Adenosine triphosphate, page 30

BAT Brown Adipose Tissue, page 36

BMI Body Mass Index, page 24

BW Body weight, page 49

CCK Cholecystokinin, page 34

CSF Cerebrospinal fluid, page 42

FFM Fat-free mass, page 51

FM Fat mass, page 51

GLP-1 Glucagon-like peptide-1, page 34

LRa Isoform of leptin receptor involved in the transport of leptin to the CSF, page 39

LRb Isoform of leptin receptor mediating leptin action in the hypothalamus, page 38

NPY Neuropeptide Y, page 37

POMC Proopiomelanocortin, page 38

PYY Peptide YY (peptide tyrosine tyrosine), page 34

WAT White Adipose Tissue, page 36

# Chapter I

# Introduction: body weight and food intake dynamics

In the context of an increasing number of overweight and obese individuals all over the world, mechanisms behind the regulation of body weight have been widely studied experimentally and theoretically in the past 25 years. This regulation is performed by the regulation of components of the energy balance: energy intake and energy expenditure. The identification of hormones enhancing or inhibiting food intake, such as leptin in 1994 [Zhang et al., 1994] and ghrelin in 1999 [Higgins et al., 2007], opened new directions for understanding and potentially controlling body weight regulation mechanisms. Understanding and targeting the origins of body weight dysregulations is important for public health. Due to the limitations in experimental approaches, in particular in humans, interdisciplinary approaches such as mathematical and computational modeling can complement experiments, assist therapeutic interventions (diet manipulation, surgery and/or drugs) or guide future experiments. Here, I will present a mathematical modeling approach of the regulation of food intake and body weight, based on biological assumptions.

In a first section, I will present the mechanisms regulating food intake and energy expenditure. I will particularly focus on action of hormonal regulators such as ghrelin, insulin and leptin, as these will be the main factors described in the models presented in the following chapters. Moreover, the main hormone known to regulate food intake and energy expenditure is leptin, acting through leptin receptors. I will then detail its actions and the links between obesity and leptin resistance.

The use of mathematical models describing the regulation of body weight and body com-

position has been increasing in the past years. In particular they are used to predict the evolution of body weight with different diets, mostly in the context of the obesity epidemics. I will then present, in the second section, a state of the art of mathematical models of the regulation of body weight, based on different biological assumptions, such as energy balance, macronutrient dynamics or leptin mediated regulation of food intake.

The third section of this chapter will consist in an introduction of the thesis work, which is presented in details in Chapters II, III and IV. I will present mathematical models of the regulation of food intake and body weight by hormones ghrelin and leptin. This model will first be applied to caloric restrictions, then to leptin resistance. A model combining both aspects will then be presented in the last chapter.

## I.1 Regulation of food intake and body weight in mammals

Body weight is highly regulated in mammals: in humans, body weight remains usually constant in adults while in rodents, body weight slightly increases during the whole life. A normal body weight can be defined as a body weight which maximizes life expectancy [Friedman, 2000]. In humans, the body-mass index [1] (BMI) gives an indication on the body weight status [World Health Organization, 2000] (see Table I.1). However, BMI is a highly simplified indicator, which fails in the cases of extreme heights and important muscle mass and is not adapted for children and elders.

Body weight can be divided into fat mass, corresponding to the lipid content of the body and fat-free mass, which represents the difference between body weight and fat mass, including muscles, bones and tissues. Fat mass usually represents 15% to 25% of body weight in humans (with higher percentages in women than in men), and can reach more than 30% in obese individuals [Friedman, 2000]. The precise measure of body fat content is not easily accessible, so BMI and waist circumference are widely used to characterize obesity. The amount of fat tissue maintained during adult life is determined by complex interactions between genetic and environmental conditions and can change if the environment changes [Barsh et al., 2000; Friedman and Halaas, 1998; Woods et al., 2000]. Dysregulations of body weight are characterized by an excessive (obesity) or a reduced body weight (cachexia, anorexia nervosa), and are associated with increased

1. the body-mass index is calculated using the formula: BMI=(body weight in kg)/(height in m)$^2$

| Body mass index | Body weight status |
|---|---|
| BMI $< 18.5$ | underweight |
| $18.5 \leq$ BMI $< 25$ | normal |
| $25 \leq$ BMI $< 30$ | overweight |
| BMI $> 30$ | obese |

**Table I.1** – Relationship between body mass index (BMI) and body weight status [World Health Organization, 2000].

morbidity. More precisely, obesity is characterized by an excessive fat mass [Atkinson, 2014] while cachexia and anorexia nervosa are characterized by low body weights due to infectious diseases or cancers for cachexia or to an eating disorder for anorexia nervosa. A large number of genes, whose allelic variations can impact body weight regulation, have been identified [Barsh et al., 2000].

Causes of body weight dysregulation include genetic and environmental factors. In rodents, some strains have mutations in genes involved in the regulation of food consumption, they cannot regulate their food consumption to maintain a healthy body weight and become obese. Most rodents submitted to a high fat diet quickly become obese, but there exist some exceptions, such as rodents able to increase their energy expenditure or strains resistant to obesity. For example, Lou/C rats, a strain of rats developed from Wistar rats [1], are obesity-resistant. Compared to Wistar rats, Lou/C rats display higher metabolic rate, physical activity and energy expenditure corrected by body mass, as well as a reduced adiposity (less adipocytes with a reduced mean size) [Soulage et al., 2008].

Obesity in humans is widely seen as a behavioral problem, characterized by a lack of discipline [Atkinson, 2014; Friedman, 2000]. The prevalence of obesity in men and women has increased in the last 30 years worldwide [Ng et al., 2014] (see Figure I.1). This disease is associated with an increased risk of developing various health problems, such as type 2 diabetes, hypertension, cardiovascular diseases, osteoarthritis and certain types of cancer [World Health Organization, 2000]. Obese people are often told that they just need to reduce their caloric intake and/or increase their physical activity to lose weight; this solution fails in most cases and people regain the lost weight [Friedman, 2000]. There exist almost no drugs treating obesity and they have limited results and usually important side effects. Obesity surgery, which consists in a reduction of the stomach, is more efficient as it induces a mechanical reduction of food intake, leading to an important decrease in body

1. Strain of albinos laboratory rats, belonging to the species *Rattus norvegicus*. Wistar rats are widely used in research as a model organism and spontaneously develop obesity when aging. Sprague-Dawley rats, Lou/C rats and Long-Evans rats were developed from Wistar rats.

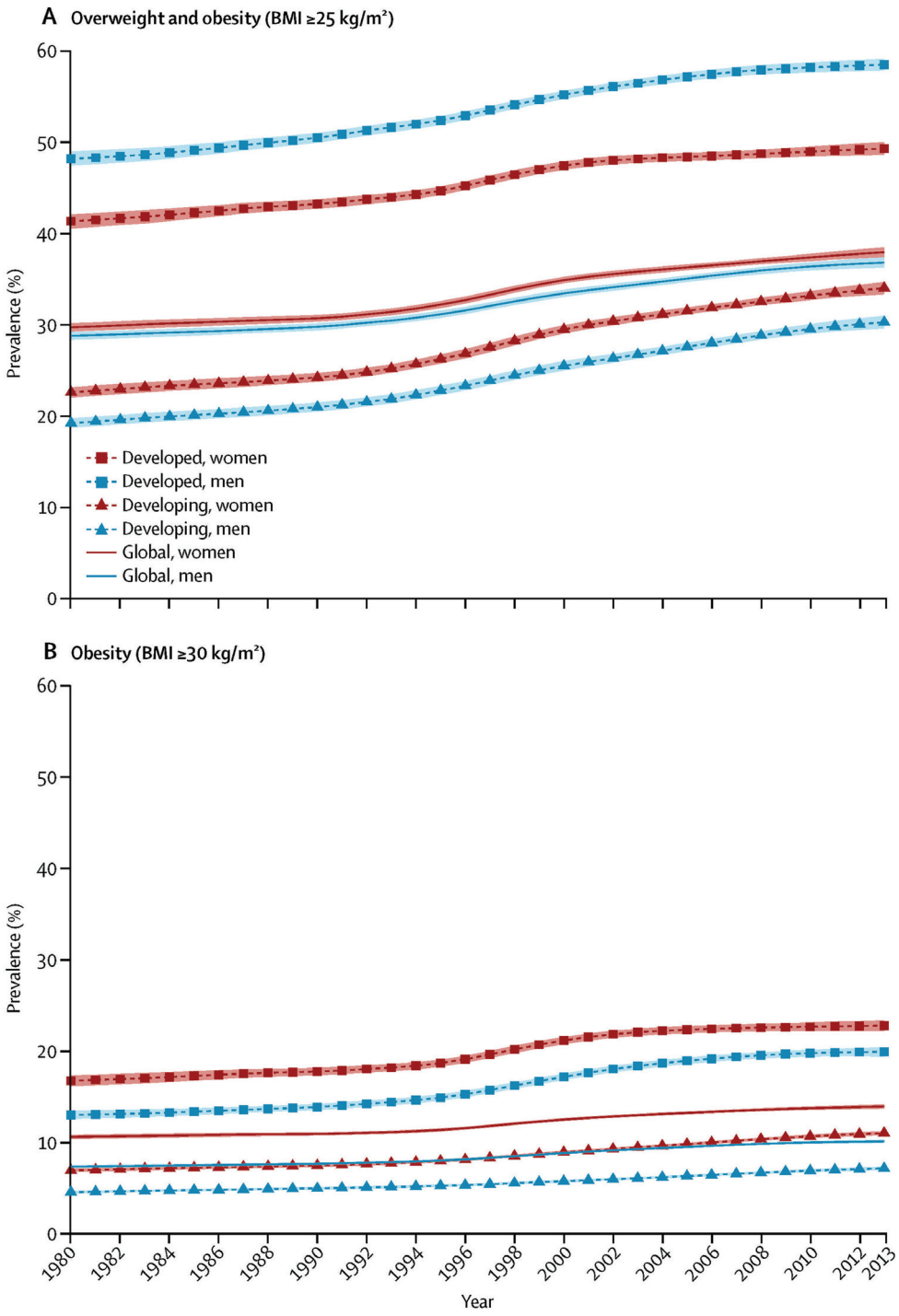


**Figure I.1** – Evolution of the prevalence of overweight and obesity in adults from 1980 to 2013. Reprinted from [Ng et al., 2014], with permission from Elsevier `http://www.elsevier.com`. Men are represented by a blue curve and women by a red curve, with a distinction between developed (squares) and developing countries (triangles). The prevalence of overweight and obesity has increased since 1980 for both men and women, in developed and developing countries, as well as the prevalence of obesity only.

weight and changes in hormones production [Atkinson, 2014; Cummings and Shannon, 2003].

Adipose tissue represents the main stock of energy in the body, as it is easier to store energy in the form of fat than in the form of protein due to its low density and high caloric content. Adipose tissue is composed adipocytes, which are cells dedicated to the storage of energy with triglycerides forming droplets inside the cytoplasm of the cells. When glucose is limited, lipolysis occurs, leading to the hydrolization of triglycerides and their extracellular release in the form of glycerol and free fatty acids, which are latter used to produce energy. Adipocytes are involved in many homeostatic processes through the synthesis and release of hormones, including leptin. Mammals, birds, reptiles and amphibians possess fat tissues composed of adipocytes, but localized in different parts of the body [Rosen and Spiegelman, 2006]. These fat depots have different impacts on health depending on their localization. There exists a distinction between white adipocytes (in the white adipose tissue), which represent the majority of adipocytes, and brown adipocytes that are involved in thermogenesis. Brown adipocytes form a distinct adipose tissue in rodents, while in humans brown adipocytes are scattered within white adipose tissue [Rosen and Spiegelman, 2006].

In the case of a body weight remaining almost constant, the number of adipocytes remains constant during adult life, with a rate of pre-adipocytes recruitment equal to the rate of dying. During weight loss, only the volume of adipocytes is changing, along with changes in lipolysis and lipogenesis [Arner and Spalding, 2010; Rossmeislová et al., 2013]. Hyperplasia (increase in adipocytes number) is sometimes observed in severe obesity. An increase in mean adipocyte size may precede the increase in adipocytes number, but fat cell count does not seem to decrease in adults [Arner and Spalding, 2010]. Adipocyte size distribution has been shown to be bimodal, with peaks around $15\mu$m and $60\mu$m [Kaplan et al., 1980; Soula et al., 2013, 2015]. Caloric restriction induces a shift in the adipocyte size distribution to smaller adipocytes [Soula et al., 2015].

Due to the constancy in body weight observed in adult humans, the existence of a set-point (target value) for body weight has been hypothesized. Based on its characteristics each individual should have a set-point, corresponding to its ideal body weight. A control system, located in the hypothalamus, should then regulate the body weight to maintain body weight around the set-point: artificial changes in body weight would be compensated to return quickly to the body weight set-point [Keesey and Hirvonen, 1997]. Perturbations or changes in environmental conditions can induce dysregulations and body weight can tend to another value, the settling-point. The settling-point results from a feedback

mechanism on body weight dependent on internal and external factors, such as genetics, epigenetics, viral infections, gut bacteria or psychology [Atkinson, 2014; Wirtshafter and Davis, 1977]. The existence of a settling-point would explain why animals submitted, for example, to hypothalamic lesions or to high-fat diets display changes in body weight. This can also be seen as a change in the body weight set-point, for example obese humans maintain a higher body weight than lean individuals, due to an increased set-point [Keesey and Hirvonen, 1997].

Body weight is controlled by modulating food consumption and energy expenditure, in response to short and long-term signals, in order to remain at an equilibrium between energy intake, corresponding to the energy absorbed from food, and energy expenditure. In young humans, energy intake exceeds energy expenditure to allow growth. During rats development, different phases of energy intake and energy expenditure appear: from month 1 to month 3, rats rapidly grow and store lipids and protein, this growth slows until month 6, during this period excessive energy is stored only in the form of lipids [Iossa et al., 1999]. Energy intake and energy expenditure keep decreasing from 1 to 6 months of age, probably due to changes in hormonal concentrations [Iossa et al., 1999].

In the following sections, I will introduce general information on the regulation of food intake and energy expenditure, and then focus on hormones. Hormones, such as ghrelin, insulin, leptin and gut hormones, are major regulators of body weight as they influence food intake and energy expenditure. Among them, leptin has probably a central impact on food intake and leptin resistance, a state of reduced responsiveness to leptin, is correlated with obesity.

### I.1.1 Regulation of food intake and energy expenditure

Variations in body weight depend on variations in energy balance, defined as the difference between energy intake and energy expenditure. Thus, regulation of body weight is directly linked to the regulation of food intake: food consumption is reduced in order to avoid gain of body weight or increased to avoid losing body weight. Energy expenditure is regulated to avoid changes in body weight: it decreases when food intake is reduced to limit the loss of body weight and increases when there is overconsumption of food [Atkinson, 2014; Friedman, 2000]. Regulation tends to match energy intake and energy expenditure on the long-term (energy homeostasis) to minimize the impact of short-term fluctuations in energy balance on fat mass [Morton et al., 2014; Woods et al., 1998, 2000]. In humans,

important daily variations in energy intake are observed, with compensations occurring at longer time scales. These variations define patterns in food intake that are correlated with BMI [Periwal and Chow, 2006]. The range of adaptation of food intake and energy expenditure is limited and all excess or deficits cannot be compensated.

Food intake is regulated in the brain by integration of signals from the rest of the body, through peripheral nerves or directly through receptors in the brain [Woods et al., 1998]. Many factors influence feeding behavior, such as emotions, olfaction or vision [Friedman, 2000]. Some signals are generated in response to food consumption while others concern the general state of the body. When food is consumed captors in the mouth, stomach and gut transmit information about the volume and composition of the food. At the same time, gut and stomach produce some orexigenic [1] or anorexigenic [2] hormones such as ghrelin, cholecystokinine and peptide tyrosine tyrosine [Guyenet and Schwartz, 2012]. In vertebrates, storage of energy is performed when food is available and reserves are depleted in the case of caloric restriction [Woods et al., 2000]. If an individual is submitted to caloric restrictions (voluntarily or not), it loses body weight, but, when food consumption is restored, its food intake will increase to restore the previous body weight. Similarly, in the case of overfeeding, body weight increases, and if overfeeding stops, the food intake decreases to return to the initial weight. This regulation of food intake is associated with changes in energy expenditure [Woods et al., 2000].

Energy expenditure can be separated into different components, including resting metabolic rate, thermic effect of feeding, adaptive thermogenesis and physical activity. Resting metabolic rate represents approximately 60% of the total energy expenditure and corresponds to essential metabolic processes [Leibel et al., 1995]. The thermic effect of feeding accounts for 10% of the total energy and corresponds to the cost of digesting and using the nutrients [Leibel et al., 1995]. Resting energy expenditure [3], in the absence of intense physical activity, is determined mainly by fat-free mass [Garrow, 1987; Nelson et al., 1992]. Obese individuals have a higher resting metabolic rate than lean individuals, due to their increased fat mass and fat-free mass [Leibel et al., 1995]. As energy expenditure is difficult to measure in humans, multiple formulas exist to predict resting energy expenditure from body weight or composition, most of them linear in fat-free mass and fat mass, sometimes with corrections on age, height or sex [Livingston and Kohlstadt, 2005; Nelson et al., 1992].

1. orexigenic: which induces food intake
2. anorexigenic: which inhibits food intake
3. Amount of energy expended at rest, similar to basal metabolic rate which is the resting energy expenditure measured just after awakening

Body weight change is submitted to multiple feedback loops. In particular, it has been shown that global energy expenditure decreases during an important caloric restriction in obese individuals. This reduction in energy expenditure can reach 30% and impact the ability to lose weight: the decrease in body weight is lower than expected knowing the reduction in caloric intake [Bray, 1969]. Weight gain in obese or lean individuals, as well as weight loss, induces changes in energy expenditure: total energy expenditure, basal metabolic rate and thermic effect of feeding increase significantly during weight gain. There exists a maximal amplitude of adaptation during weight gain and weight loss, and further changes will not be compensated [Leibel et al., 1995]. This adaptation in energy expenditure will be necessary to explain experimental data and will be considered in Chapter II [Jacquier et al., 2014]. The percentage of energy expenditure variation is similar for the same degree of underfeeding and overfeeding [Garrow, 1987]. The change in energy expenditure during weight gain or loss is more important than expected from the change in body composition, indicating other forms of regulation such as adaptive thermogenesis, probably due to hormonal fluctuations and changes in fat stores [Doucet et al., 2001; Dulloo and Jacquet, 1998; Rosenbaum and Leibel, 2010; Tremblay et al., 2013].

Adaptive thermogenesis corresponds primarily to the production of heat in response to environmental condition or diet [Lowell and Spiegelman, 2000]. The reduction in thermogenesis induced by starvation persists after the end of the period of starvation, improving the replenishment of fat stores [Dulloo and Jacquet, 1998]. Thermogenesis has then an impact on the ability of individuals to lose body fat and to maintain a reduced body weight after a weight loss program. Adaptive thermogenesis occurs mainly in the brown adipose tissue in rodents, with a regulation in the hypothalamus involving leptin and thyroid hormone. Humans do not have as much brown adipose tissue as rodents, but their skeletal muscles are involved in thermogenesis. Increased thermogenesis is linked to waste of ATP [1] in futile cycles in the mitochondries [Lowell and Spiegelman, 2000]. Ambient temperature can then have an impact on the ability to lose weight: in rats, body weight loss induced by caloric restriction is more important in a cool environment than at thermoneutrality (approximately 30°C) [Evans et al., 2005].

Aging impacts body weight regulation, as it is associated with changes in energy expenditure, hormonal production and sensitivity. Wistar rats spontaneously develop moderate obesity when aging with increasing body weight and percentage of body fat [Newby et al., 1990]. This progressive accumulation of lipids is associated with increased number and

1. Adenosine triphosphate, used a an energy source for cellular functions

size of adipocytes. Metabolic rate is highest in young rats then decreases (in two steps, one important decrease followed by a progressive decrease) then slightly increases during the last months of life. The increase in metabolic rate observed in old rats is associated with age-related diseases [McCarter and Palmer, 1992].

The regulation of body weight, resulting from the regulation of food intake and energy expenditure, is mediated in the central nervous system, mainly in the hypothalamus, by integrating afferent signals from the body. Lesions in different hypothalamic regions have been shown to dysregulate body weight, locating body weight regulating system in these regions [Elmquist et al., 1999]. These signals correspond to substances in blood (nutrients or hormones) but also nervous signals from the gut, informing the brain on the state of the energy reserves and the recent food consumption. Hormonal signals include ghrelin, leptin, insulin and gut hormones [Morton et al., 2014] (see Figure I.2 for a summary of the food intake regulation system and Table I.2). The system will then adapt to the situation by increasing or decreasing food intake and energy expenditure. Adaptation of energy expenditure occurs through modulation of physical activity, resting metabolic rate and thermogenesis. In humans, this allows, on the long term, to maintain an equilibrium between energy intake and energy expenditure, despite differences at low time scales [Morton et al., 2006; Schwartz et al., 2000]. The concept of an adiposity negative feedback involved in body weight regulation was introduced 60 years ago [Morton et al., 2006], as follows:

- the signal circulates proportionally to fat mass and enters the brain,
- it acts on neuronal systems implicated in energy homeostasis and promotes weight loss,
- stopping these neuronal actions increases food intake and body weight.

Only hormones leptin and insulin fulfill all these criteria and can be considered as adiposity feedback signals [Morton et al., 2006].

Ghrelin, gut hormones and insulin are presented below. These hormones will be modeled as regulators of food intake in the work presented in Chapter II. Another hormone, leptin, is considered to be a central regulator of food intake, as an adiposity signal. The role of leptin will be presented in details in Section I.1.3.

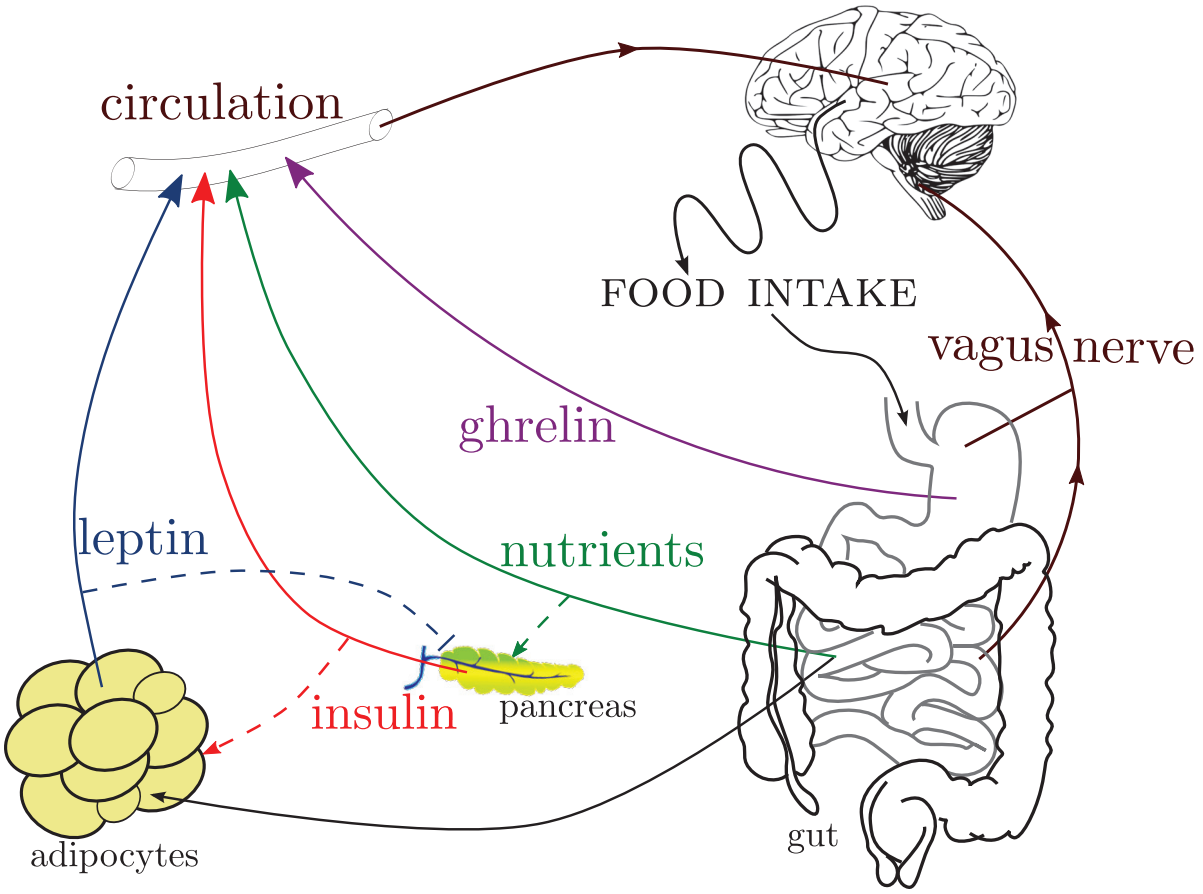


**Figure I.2 –** Schematic representation of food intake regulation. Colored arrows indicate a production, dashed bar-headed lines represent a negative influence on a production while dashed arrows represent a positive influence on a production. Food intake induces changes in the stomach which produces ghrelin (in purple), an enhancer of food intake, and in the intestine which produces gut hormones. The vagus nerve transmits mechanical and chemical signals (due to gut hormones) to the brain to reduce food consumption. Food intake leads to increased fat stores, which produce leptin (in blue); this production is increased by insulin (in red) produced by the pancreas in response to plasma glucose (nutrients) and inhibited by leptin.
Leptin, insulin, ghrelin and nutrients, such as free fatty acids or glucose join the circulation and cross the blood-brain barrier, to reach the brain. The hypothalamus (in particular the arcuate nucleus) integrates all signals to regulate food intake.

| Hormone | Production | Function |
|---|---|---|
| Ghrelin | stomach<br>(depending on food intake) | – orexigenic<br>– short time scale |
| Gut hormones | digestive system | – anorexigenic<br>– short time scale |
| Insulin | pancreatic $\beta$-cells<br>(in response to plasma glucose) | – anorexigenic<br>– regulation of plasma glucose<br>– upregulation of leptin production |
| Leptin | adipocytes<br>(proportionally to fat mass) | – anorexigenic<br>– reduction of energy expenditure<br>– downregulation of insulin production<br>– long time scale |

**Table I.2** – Summary of the characteristics and functions of hormones involved in the regulation of body weight

### I.1.2 Hormonal regulators of food intake: ghrelin, insulin and gut hormones

Ghrelin is an hormone produced mainly in the stomach, and in lower quantities in the small intestine [Crespo et al., 2014; Cummings and Shannon, 2003]. Ghrelin concentration increases when fasting (in humans, the concentration of ghrelin is maximal before meals) and its production is inhibited when the stomach is full, depending on the amount and macronutrient composition of the food [Cummings, 2006]. Ghrelin thus acts as a signal of a full stomach. In rats, a high-fat diet leads to lower ghrelin concentrations [Beck et al., 2002]. In humans, meals rich in carbohydrates induce the most important decrease in ghrelin concentration [Erdmann et al., 2004].

Ghrelin is a peripheral signal, with a short 24 min half-life [Vestergaard et al., 2007], that triggers food intake via hypothalamic neurons in the arcuate nucleus [1]. Ghrelin is the only known circulating orexigenic hormone, and an injection of ghrelin in the blood or in the cerebrospinal fluid leads to increased food intake [Cummings and Shannon, 2003; Cummings, 2006; Higgins et al., 2007; Wren et al., 2001]. Ghrelin may play a role in meal initiation, an injection of ghrelin induces eating in rodents and its levels are highest just before meals [Cummings and Shannon, 2003; Higgins et al., 2007]. In rodents, ghrelin induces a decrease in fat utilization, promoting adiposity and weight gain [Tschöp et al., 2000] but ghrelin concentrations are negatively correlated to the degree of obesity [Beck

1. Group of neurons in the hypothalamus, in particular neuroendocrine and centrally-projecting neurons.

et al., 2002; Cummings and Shannon, 2003]. Conversely, fasting and caloric restrictions induce an increase in ghrelin levels [Reimer et al., 2010]. There exist an inverse relationship between insulin and ghrelin levels: insulin has an inhibitory role on ghrelin production [Erdmann et al., 2004, 2005]. Leptin also inhibits ghrelin production for moderate gains in body weight [Erdmann et al., 2005]. These phenomena could correspond to a feedback loop to limit energy intake.

Insulin is another important hormone involved in the regulation of food intake. Insulin is produced by pancreatic $\beta$-cells, in the islets of Langerhans, in response to high levels of glucose in the blood. Insulin regulates the concentration of glucose in the blood by increasing glycogen synthesis [1] and uptake of blood glucose by muscles or fat tissue, and decreasing the conversion of non-carbohydrate substrate into glucose (for example transformation of lipids into glucose in adipocytes). Thus, insulin and glucose concentrations in blood are highly correlated, with similar variations slightly delayed, except in the case of diabetes mellitus. Type 1 diabetes mellitus is an auto-immune disease resulting in the destruction of pancreatic $\beta$-cells [World Health Organization, 1999]. The production of insulin is then very low and the only way to maintain a normal blood sugar level is to compensate this lack by injection of exogenous insulin. Type 2 diabetes mellitus is often a consequence of obesity, it is characterized by a high level of blood sugar and insulin resistance [World Health Organization, 1999], with limited changes in the amount of pancreatic $\beta$-cells. It thus cannot be treated by injection of insulin, only by changes in diet and some other medication. Insulin acts on adipocytes in the white adipose tissue to regulate leptin production, increased insulin concentration induces an increased leptin gene expression and protein secretion [Margetic et al., 2002]. Leptin receptors are expressed in pancreatic $\beta$-cells where it can impact insulin production. Circulating insulin is proportional to adiposity and enters the central nervous system by a receptor-mediated saturable transport, similarly to leptin [Woods et al., 1998]. Insulin was the first hormone found to interact with neurons from the arcuate nucleus of the hypothalamus to reduce food intake [Crespo et al., 2014; Schwartz et al., 2000].

Gut hormones, in particular cholecystokinin (CCK), peptide tyrosine tyrosine (peptide YY or PYY) and glucagon-like peptide-1 (GLP-1), are anorexigenic hormones. These hormones are produced in reaction to food consumed as it goes through the digestive system. CCK, produced by the small intestine after the meals, induces a reduction of food intake depending on intestinal nutrients, in particular fats and proteins [Covasa, 2010; Crespo et al., 2014; Duca and Covasa, 2012]. This hormone acts, at a short-term, by reducing

1. storage of glucose by the liver in the form of glycogen

meal size, in the central nervous system but also on the pancreas and the stomach. It has been shown that a high-fat diet leads to a reduced sensitivity to CCK [Duca et al., 2013]. Peptide YY has been detected in increasing quantities in enteroendocrine cells from the stomach to the colon and is released proportionally to caloric intake shortly after the meals and during a few hours [Crespo et al., 2014; Duca and Covasa, 2012]. This hormone has an anorexigenic effect mediated in the arcuate nucleus. Chronic injection of peptide YY leads in animals to a decrease in body weight, making it a potential treatment in obesity [Duca and Covasa, 2012]. GLP-1 derives from proglucagon, produced in pancreatic $\alpha$-cells, in the gut and in the brain stem, and is secreted in the circulation after a meal in response to nutrients and its concentration remains elevated for a few hours. GLP-1 acts locally by activating vagal afferents and in the central nervous system to decrease food intake without promoting satiety. GLP-1 also interacts with other hormones controlling food intake, such as leptin [Crespo et al., 2014; Duca and Covasa, 2012]. These meal-related satiety signals have a limited impact on adiposity, with an action at the time scale of the meal [Woods et al., 1998]. Brain sensitivity to these signals, generated in response to food consumed, are partly determined by adiposity signals such as leptin [Woods et al., 2000].

Ghrelin, insulin and gut hormones are important regulators of food intake, in particular ghrelin which is the only known orexigenic hormone. However, leptin is central in the regulation of food intake as it is an indicator of energy storage in fat tissue and will be the main regulator considered in this work.

## I.1.3 Leptin and leptin resistance

### I.1.3.1 Leptin

I will now present leptin and its actions on food intake and multiple physiological systems of the body. Leptin is the main anorexigenic hormone, acting in the central nervous system to regulate food intake and energy expenditure but also on peripheral tissues to regulate other processes [Schwartz et al., 2000]. It is an indicator of the fat reserves of the organism, as it is produced by adipocytes in white adipose tissue proportionally to fat mass [Auwerx and Staels, 1998]. The production of leptin by each adipocyte correlates with its lipid content and size [Friedman and Halaas, 1998]. It is also produced in low quantities by brown adipose tissue and in negligible quantities by other organs. Leptin is mainly eliminated from the blood via renal elimination [Zeng et al., 1997]. In humans, plasma

leptin levels show circadian variations with a nocturnal peak [Mantzoros, 1999]. Fasting induces an important decrease in plasma leptin, even before significant changes occur in fat mass [Friedman, 1998]. Leptin levels in women are higher than in men, and this cannot be explained only by the higher percentage of fat mass in women [DePaoli, 2014]. In mice, leptin injection induces a reduction in food intake but energy expenditure remains constant despite the reduced food intake: leptin prevents the energy expenditure from decreasing [Halaas et al., 1997]. Leptin action tends to preserve fat stores of the organism instead of depleting them [Rosenbaum and Leibel, 2014]. Leptin could be responsible for the progressive decline in energy intake observed during growth, as fat mass constantly increases during development. The constant accumulation of fat during adult life in rats could result from a decreasing sensitivity to leptin [Iossa et al., 1999].

In addition to its effect on the regulation of energy intake and energy expenditure, leptin has an action on multiple tissues in the body including regulation of arterial pressure, immunity, secretion of thyroid and sexual hormones and hematopoiesis (see Figure I.3). Leptin, via the activation of leptin receptors in hematopoietic stem cells, provides a proliferative signal for hematopoiesis, resulting in increased myelopoiesis, erythropoiesis and lymphopoiesis [Bennett et al., 1996]. Constant leptin infusion in rats induces an increase in arterial pressure and heart rate, this phenomenon is regulated in the central nervous system and could result from the control of renal function by leptin [Correia et al., 2001; Shek et al., 1998]. Leptin plays an important role in reproduction, with leptin receptors located in ovaries, prostate and placenta; it impacts fetal growth and metabolism and could also have an impact on the onset of puberty [Margetic et al., 2002]. Male and female *ob/ob* mice [1], which lack functional leptin, are sterile, but injections of leptin can restore their fertility [Auwerx and Staels, 1998; Margetic et al., 2002]. Leptin also impacts glucose transport, with increased uptake of glucose in skeletal muscles independently of insulin [Margetic et al., 2002]. Leptin, via the activation of leptin receptors, induces changes in gene expression in brown adipose tissue (BAT) and white adipose tissue (WAT), leading to an increased glucose utilization in BAT and an increased lipolysis in WAT [Siegrist-Kaiser et al., 1997; Wang et al., 1999]. Leptin induces an autocrine negative regulation of leptin gene expression in adipocytes [Zhang et al., 1997]. Leptin receptors also mediate the action of leptin on pancreatic $\beta$-cells, where it inhibits basal insulin release [Emilsson et al., 1997]. These effects of leptin on different systems of the body could explain some health problems observed in leptin/leptin receptors mutant individuals (rodents or humans), such as hyperphagia, reduced immunity, hypothyroidie or fertility issues, the individual is in a state of "perceived starvation" [Friedman and Halaas, 1998]. When this

1. Strain of obese mice, characterized by a recessive mutation on the gene *ob* coding for leptin.

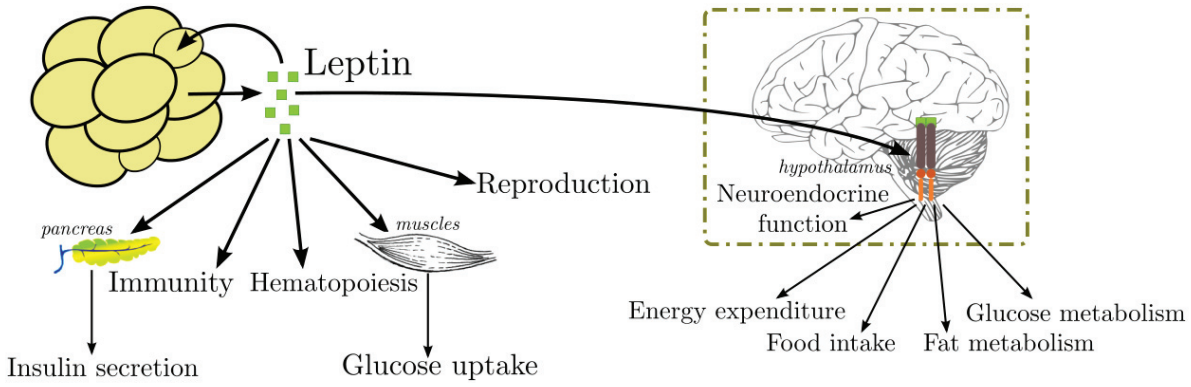


**Figure I.3 –** Representation of some central and peripheric actions of leptin.

mutation induces leptin deficiency, a treatment by injection of leptin can normalize the situation [Farooqi and O'Rahilly, 2014].

Leptin (from the Greek $\lambda\epsilon\pi\tau\acute{o}\varsigma$, which means "thin"), originally known as the "obese gene product", was discovered in 1994 [Friedman, 2014; Zhang et al., 1994]. Its existence as a circulating factor regulating food intake and body weight had been hypothesized before due to parabiosis [1] experiments on *ob/ob* mice, whose body weight partially returned to normal after being connected to healthy mice [Zhang et al., 1994]. The same experiment conducted on *db/db* mice did not have any impact, as these mutant mice produce functional leptin but are lacking leptin receptors [Friedman, 1998; Halaas and Friedman, 1997; Tartaglia, 1997]. Leptin gene is highly conserved among vertebrates, in particular the predicted amino-acid sequence is 84% identical between mice and humans, suggesting a highly conserved function [Zhang et al., 1994]. However, leptin has not been detected in invertebrates unlike insulin, indicating a more recent evolution [Morton et al., 2006].

In the hypothalamus, the action of leptin on food intake and energy expenditure is mediated via the regulation of various neuropeptides gene expression and release [Sahu et al., 2001]. Leptin induces a downregulation of orexigenic neuropeptides, such as neuropeptide Y or orexins and an upregulation of anorexigenic neuropeptides, such as neurotensin, cocaine and amphetamine regulated transcript (CART) or melanocyte-stimulating hormone (MSH) [Elmquist et al., 1999; Friedman and Halaas, 1998; Woods et al., 2000].

Leptin induces a downregulation of neuropeptide Y (NPY) synthesis and release in the arcuate nucleus, by acting on NPY neurons. The action of leptin on NPY partly explains the effect of leptin on food intake and energy expenditure [Crespo et al., 2014; Schwartz et al., 1996b; Stephens et al., 1995]. Ghrelin also acts on NPY neurons, with opposite

1. partial connection of circulatory systems of animals

effects as leptin [Higgins et al., 2007; Tschöp et al., 2000]. This neuropeptide is the most potent known orexigenic agent and is found in high concentration in the areas of the hypothalamus involved in the regulation of feeding behavior. NPY induces an important increase in food intake when injected into the hypothalamus of rats [Sahu and Kalra, 1993]. NPY also induces a decrease in thermogenesis and an increase in plasma insulin [Stephens et al., 1995]. Fasting, on the other hand, stimulates NPY gene expression [Schwartz et al., 1996b]. NPY neurons inhibit proopiomelanocortin neurons, also involved in food intake regulation [Morton et al., 2006].

Leptin also promotes the production of anorexigenic neuropeptides. Leptin acts on proopiomelanocortin (POMC) neurons in the arcuate nucleus, inducing an increase in POMC. POMC is a precursor for melanocortins, which are anorexigenic neuropeptides, in particular $\alpha$-MSH. Melanocortins then bind and activate melanocortin receptors to decrease food intake. As for NPY, the POMC mediated pathway is modulated by high and low leptin [Schwartz et al., 2000; Woods et al., 2000]. Effects on POMC neurons, unlike NPY neurons, do not occur rapidly, suggesting an action on the long-term control of feeding [Morton et al., 2014]. Leptin induces an increase in CART levels; this peptide then induce a suppression of feeding [Elmquist et al., 1999].

In lean animals, the injection of leptin induces a dose-dependent decrease in body weight, in the form of a loss of fat mass which can result in a complete depletion of fat stores. This high concentration of leptin, allows the metabolic rate to remain high, unlike caloric restriction, which is associated with a reduced metabolic rate [Woods et al., 1998].

In this work I will consider the regulation of food intake by leptin without introducing the variety of neuropeptides involved in this regulation. In order to regulate food intake and energy expenditure in the arcuate nucleus of the hypothalamus, leptin has to cross the blood-brain barrier and then activate specific receptors. The transport to the brain is performed by a saturable system, probably involving leptin receptors instead of diffusion through the blood brain-barrier [Banks et al., 1996, 2000b; Banks, 2004]. Leptin receptors are then necessary for leptin action and are regulated by leptin.

#### I.1.3.2 Leptin receptors

The action on leptin in the hypothalamus occurs via specific receptors: LRb. When leptin binds to its specific receptors, it induces a cascade of reactions resulting in a downregulation of food intake.

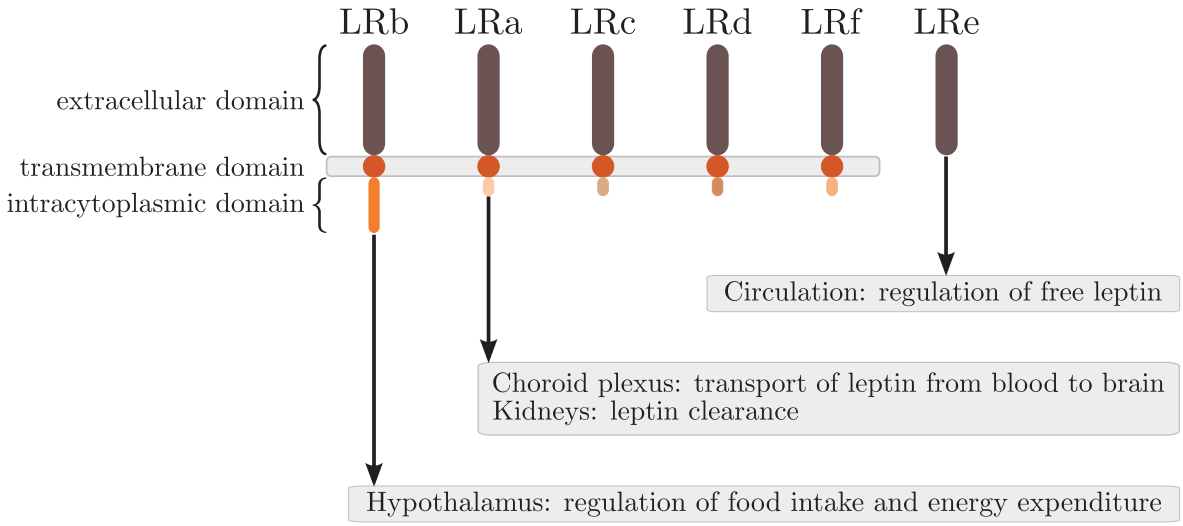


**Figure I.4** – Leptin receptor (LR) isoforms associated with their main localization and role.

Leptin receptors belong to the class I cytokine receptors family. They are the result of alternative splicing of leptin receptor gene, previously known as *db* gene [Peelman et al., 2014; Tartaglia, 1997]. Animals lacking functional leptin receptors, such as *db/db* mice and *fa* rats, develop obesity. There exist 6 isoforms of receptors characterized by their intracellular domains: LRa to LRf. All receptors, except LRe which is secreted in the blood in mice, have the same extracellular and transmembrane domains (see Figure I.4). LRb receptors have a long intracytoplasmic domain, LRe receptors have no intracytoplasmic domain and the 4 remaining types of receptors (LRa, LRc, LRd, LRf) have short intracytoplasmic domains [Peelman et al., 2014]. The signaling cascade to regulate food intake can only occur with long-form leptin receptors. Leptin and leptin receptor LRe can form a circulating complex, which may participate in the regulation of circulating leptin [Friedman and Halaas, 1998; Myers et al., 2008].

LRa receptors, previously known as OB-$R_S$ or ObRa, are the most abundant isoform of leptin receptors [Myers et al., 2008]. They are involved in the saturable transport of leptin via receptor-mediated transcytosis [1] through the hemato-encephalic barrier [Golden et al., 1997; Kastin et al., 1999; Lynn et al., 1996], as they are widely expressed in the choroid plexus [Tartaglia et al., 1995]. The choroid plexus is a component of the blood-brain barrier responsible for the production of cerebrospinal fluid. Leptin is a big protein, so it has difficulties to cross the blood-brain barrier by diffusion and it needs to be transported from the blood to the cerebrospinal fluid, in order to act on hypothalamic neurons. Rats

1. Transport of a macromolecule through the interior of the cell.

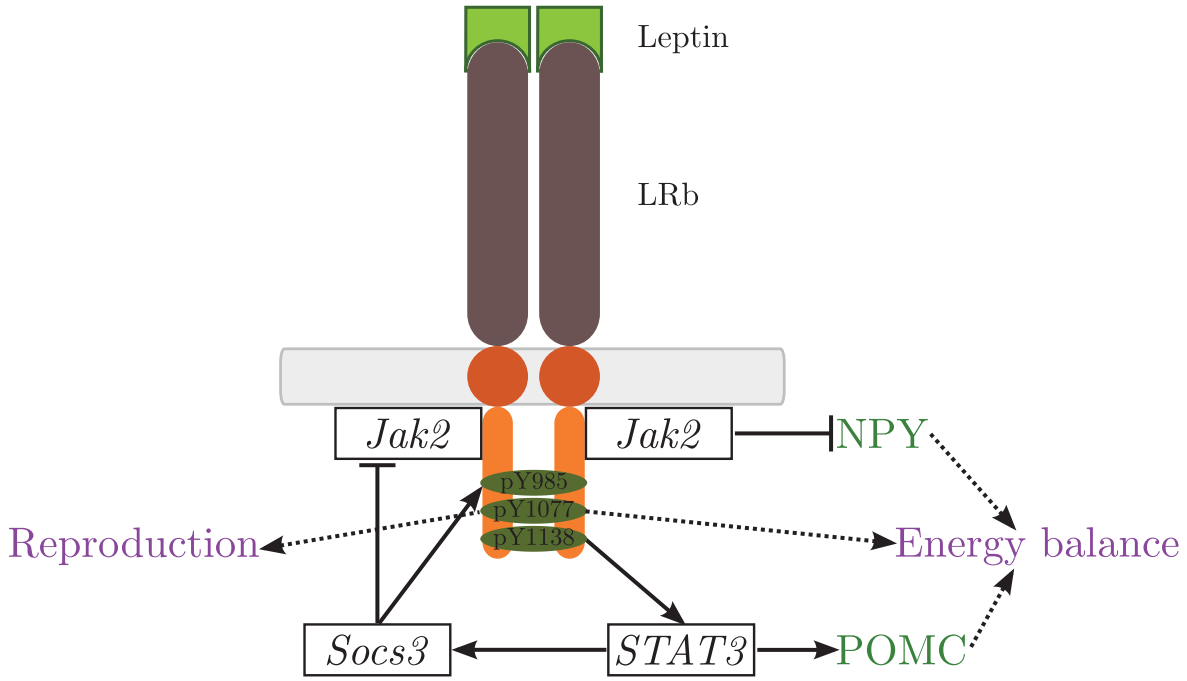


**Figure I.5** – Simplified leptin signaling after binding to LRb receptors in the hypothalamus with associated biological functions. Straight lines indicate signalling pathways, dotted lines link to the biological function.

lacking functional LRa (Koletsky rats) are obese and have significantly decreased influx of leptin from blood to brain [Kastin et al., 1999]. They are also found in other tissues, such as kidneys and lung [Tartaglia et al., 1995; Tartaglia, 1997], where they can mediate leptin action in particular leptin clearance in the kidneys [Bjørbaek et al., 1997].

LRb receptors, previously known as OB-$R_L$ or ObRb, which are highly expressed in the hypothalamus, mediate the action of leptin on food intake and energy expenditure. In the brain, these receptors are found on specific neurons, which are activated by leptin binding to LRb receptors. LRb neurons include NPY and POMC neurons in the arcuate nucleus, which are known to respond to leptin [Münzberg and Myers, 2005]. Leptin binding occurs with homo-dimerization of the receptor and activates an intracellular signaling cascade involving JAK2/STAT3 pathway [Bjørbaek et al., 1997; Tartaglia, 1997] (see Figure I.5). Another signaling pathway, involving SOCS3, results in a feedback inhibition of LRb signaling, making it a potential mechanism for leptin resistance [Bjørbaek et al., 2000]. Chronic high leptin concentration induce a downregulation of leptin receptor mRNA and protein in the hypothalamus [Martin et al., 2000]. These receptors are also found in low quantities in other organs, where they mediate leptin action other than food intake regulation. These receptors will be considered in the model of leptin resistance (Chapter

III), due to their importance in the regulation of food intake by leptin.

Leptin is a regulator of LRb receptors: an injection of exogenous leptin induces a downregulation of LRb receptors mRNA and protein expression [Martin et al., 2000]. This downregulation is also observed in obese individuals and diet-induced obese animals, which exhibit increased leptin levels and a downregulation of leptin receptors. However, caloric restriction reverses this deficit in LRb receptors and may be an efficient mechanism to restore LRb receptors levels, and thus leptin signaling in the hypothalamus. Thus, the regulation of leptin receptors by leptin leads to a regulation sensitive to leptin variations, in particular to high leptin. This downregulation of leptin receptors by leptin is a possible mechanism behind the development of leptin resistance [Wilsey and Scarpace, 2004; Zhang and Scarpace, 2006].

#### I.1.3.3 Leptin resistance and pathway to obesity

Leptin resistance corresponds to the inability of the body to respond to high concentrations of leptin in blood, thus the system does not reduce food intake at all or not as importantly as in a healthy individual. Hence, a higher leptin level will be necessary to obtain the same response. This corresponds to a state of leptin resistance, by analogy to insulin resistance. Leptin resistance is often observed in obese individuals, associated with high levels of circulating leptin [Friedman and Halaas, 1998]. It is mainly an acquired condition in humans, except for some rare gene mutations (for example mutations similar to *db/db* mutations in mice which result in a lack of functional LRb). There also exist some cases of human obesity associated with relatively low leptin levels, representing 5 to 10 % of the obese population, resulting from a reduced rate of leptin production [Friedman and Halaas, 1998].

The regulation of food intake by leptin is impacted by aging. The decrease in food intake consecutive to leptin injection is lower in old rats than in young rats [Scarpace et al., 2000; Scarpace and Zhang, 2009]. Some common strains of old laboratory rats, such as Sprague-Dawley, Wistar or F-344xBN strains, are obese, exhibit leptin resistance, impaired leptin signal transduction, have elevated leptin concentrations and reduced leptin receptors compared to young rats. This resistance to the action of leptin is either observed for both central and peripheral injection of leptin or only for peripheral injection, indicating different types of resistance in the different strains of aged-obese rats [Scarpace and Tümer, 2001; Scarpace et al., 2001]. In addition to the reduced impact of leptin on food intake, energy expenditure is not modified by leptin in aged-obese rats [Scarpace and

Tümer, 2001].

Leptin resistance can occur at different steps of the leptin regulation pathway: at the blood-brain barrier, in the hypothalamus or during the food regulatory pathway resulting from activation of neurons by leptin, depending on the organism [Martin et al., 2000; Myers et al., 2008; Zhang and Scarpace, 2006]. The resistance probably occurs at multiple levels [El-Haschimi et al., 2000]. An injection of leptin in plasma will not lead to a reduction in food intake for a leptin-resistant individual. However, if leptin is injected directly into the CSF it can induce a decrease in food intake, depending on the location of the resistance: if the resistance occurs at the blood-brain barrier only leptin injected into the CSF will impact food intake [El-Haschimi et al., 2000; Halaas et al., 1997]. In the case of hypothalamic leptin resistance, the inhibition of food intake following central leptin injection can be absent or just reduced [Widdowson et al., 1997]. This resistance explains why therapeutic use of leptin to reduce obesity does not work, as the system, if not already resistant quickly becomes leptin resistant. In the rare case of mutations on the gene encoding leptin, the injection of functional leptin leads to a regulation of food intake and consequently of body weight. Mutations in neuropeptides mediating the action of leptin, such as melanocortin, can also induce leptin resistance and obesity [Schwartz et al., 2000].

The composition of the diet, such as diets rich in fructose, can induce leptin resistance. In rats, a diet rich in fructose and fat induces high plasma leptin and leptin resistance, which is not the case for a diet with high fat but without fructose. This diet-induced leptin resistance is reversible by removing the fructose from the diet [Shapiro et al., 2011]. This leptin resistant animal models display similar body weights, fat mass and serum leptin [Scarpace and Zhang, 2009]. Elevated circulating triglycerides can also induce leptin resistance, by decreasing leptin transport through the blood brain barrier [Banks et al., 2004]. In obesity-prone rats, such as Sprague-Dawley strain, overfeeding induces important increase in leptin concentration, leptin and insulin resistance after a few days, indicating that hyperphagia can induce leptin resistance as well as leptin resistance induces hyperphagia [Wang et al., 2001].

Leptin can cause leptin resistance, as high leptin concentration induces a downregulation of leptin receptors and a decreased leptin signaling capacity in the hypothalamus, in particular in NPY neurons [Pal and Sahu, 2003; Sahu, 2002; Scarpace et al., 2005]. Chronic leptin infusion in the hypothalamus rapidly induces leptin resistance. Food intake, initially importantly reduced, starts to return to its initial level a few days after the beginning of the infusion [Pal and Sahu, 2003; Sahu, 2002]. Leptin resistance favours fat deposition

and increases the susceptibility to develop obesity, when environmental conditions are favorable to weight gain such as high-fat diet or palatable food [Scarpace et al., 2005; Scarpace and Zhang, 2009]. The regulation of leptin receptors by leptin will be the base of the work on the development of leptin resistance presented in Chapter III [Jacquier et al., 2015].

Rodents with diet-induced obesity have a significant reduction in LRb receptors in the hypothalamus, which are inhibited by leptin. This represents a possible mechanism of leptin resistance in the hypothalamus [Wilsey and Scarpace, 2004]. This reduction in receptors is reversible, if leptin levels decrease, usually after returning to a normal diet [Scarpace and Zhang, 2009]. Another possible mechanism inducing leptin resistance is due to LRb signalling: increased leptin induces a small increase in LRb signaling, this increase becomes smaller and smaller as leptin keeps increasing. In mice with diet-induced obesity, LRb signaling is slightly increased but cannot compensate the important increase in leptin levels, indicating leptin resistance [Myers et al., 2008].

It is not clear if leptin resistance is a cause, a consequence or both a cause and a consequence of obesity. Leptin resistance induces an increased susceptibility to dysregulation of food intake leading to an excessive accumulation of fat mass and obesity (diet-induced obesity) [Guyenet and Schwartz, 2012; Scarpace and Zhang, 2009; Zhang and Scarpace, 2006]. Obesity, with an increased fat mass, is often associated to high concentrations of leptin in plasma which impact leptin receptors and can induce resistance. Obesity also promotes cellular processes leading to an attenuation of leptin signaling [Myers et al., 2010]. This induces a vicious cycle of obesity promoting leptin resistance which promotes further accumulation of fat mass [Scarpace and Zhang, 2009; Zhang and Scarpace, 2006] (see Figure I.6). The primary cause of increased fat mass may be independent of leptin, such as palatability of food or actions of other hormones [Myers et al., 2010].

Some strains of obese mice show a reduced rate of leptin transport through the blood brain barrier compared to lean mice, leading to a reduced capacity to transport leptin [Banks et al., 1999]. This phenomenon is due to a defect in the transporter: the system is not able to upregulate leptin transporters leading to a decreased transport of leptin to the brain, where the regulation of caloric intake cannot occur, and increased adiposity [Banks et al., 1999] (see Figure I.7). This transporter is probably LRa leptin receptors. The defect in transport is acquired with the progression of obesity and reversible after caloric restriction and weight loss [Banks and Farrell, 2003].

In humans, the ratio in leptin concentration between the CSF and the serum decreases

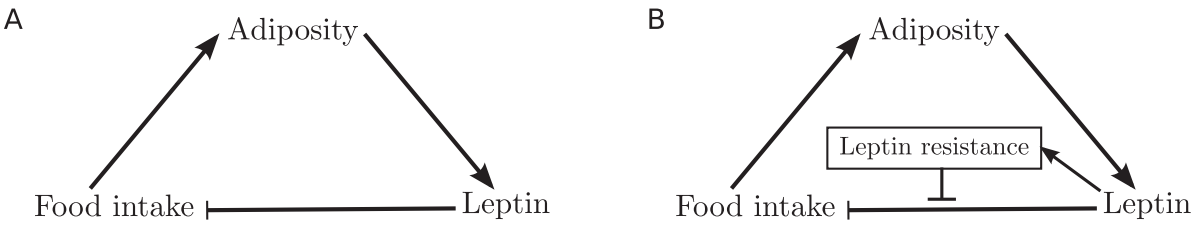


**Figure I.6 –** Regulation of food intake and body weight for normal and leptin resistant cases. A. Normal cycle of body weight regulation by leptin: increased adiposity leads to an increased leptin concentration which inhibits food intake. This leads to a reduced body weight, and the system stays at an equilibrium. B. The inhibition of food intake by leptin is disrupted in the case of leptin resistance. Increased adiposity leads to increased leptin which does not impact food intake: body weight is not regulated. As leptin can impact leptin resistance, this corresponds to a vicious cycle of increasing body weight leading to obesity.

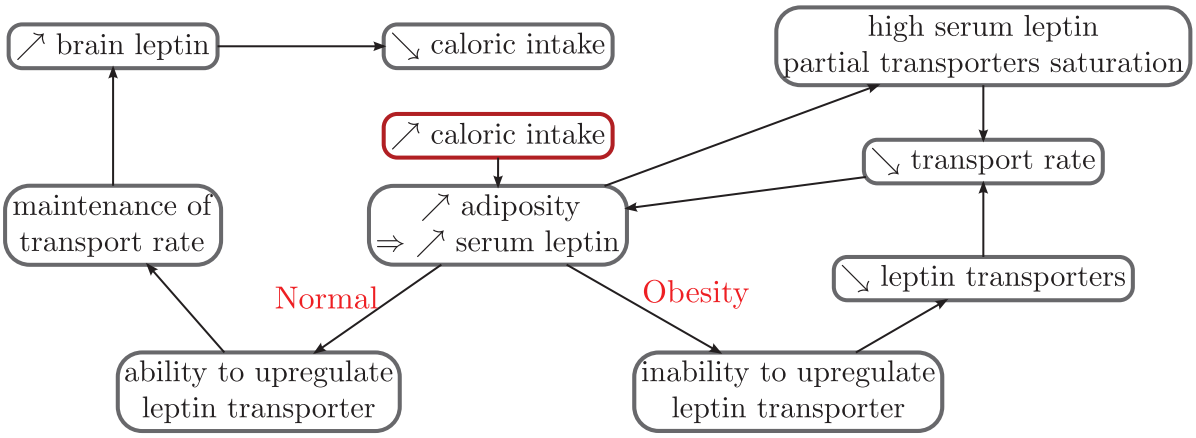


**Figure I.7 –** Representation of a model of obesity based on a defect of leptin transporter through the blood brain barrier (adapted from [Banks et al., 1999]). Normal regulation mechanisms are represented on the left, with an increase in caloric intake eventually leading to a decrease in caloric intake (normalization of the situation). On the right (obese situation), an increase in leptin induces a downregulation of transporters and an increased adiposity.

with BMI, with a reduced ratio in obese individuals [Caro et al., 1996; Schwartz et al., 1996a], suggesting a saturable transport system with reduced efficiency in obese individuals. However, leptin in CSF, as leptin in plasma, is still correlated to BMI. This reduced efficiency in leptin transport probably contributes to leptin resistance at the blood-brain barrier [Schwartz et al., 1996a].

Because of its weight reducing effect, leptin has been tested as a cure to obesity. Leptin-induced body weight loss mainly consists in fat-mass loss which is not the case for diet-induced weight loss [Mantzoros, 1999]. However, due to leptin resistance it has no effect on body weight in obese individuals, except the ones who have total or partial leptin deficiency [DePaoli, 2014]. Leptin administration during caloric restriction can mitigate hunger and help losing body weight, by compensating the decline in endogenous leptin due to the depletion of fat mass [Rosenbaum and Leibel, 2014]. Associating leptin with other molecules affecting leptin signaling pathway which could increase leptin sensitivity or reverse leptin resistance is a potential treatment for obesity, as well as the use of leptin analogues or other hormones [DePaoli, 2014; Friedman, 2014; Rosenbaum and Leibel, 2014].

Leptin is then an important regulator of food intake and energy expenditure, via the activation of specific receptors in the hypothalamus. Leptin resistance is probably a cause and a consequence of obesity and creates a favorable environment for weight gain.

Regulation of body weight, performed by regulating food intake and energy expenditure, is a complex process involving multiple pathways and an important number of molecules. Environment tends to perturb this system. In the context of the increasing number of obese people, the comprehension of regulatory mechanisms brings potential targets to manage weight loss. The regulatory mechanisms described in this part will be used in the development of mathematical models describing the dynamics of body weight and leptin resistance in Chapters II and III. Mathematical models have been developed to integrate the regulation and adaptations in energy intake and energy expenditure and help optimizing weight loss or weight gain programs, as they provide a quantitative representation of the regulation of body weight. The complexity of the regulation, with a lot of interacting components, can then be included in the models, and allows to test the effect of experimentally impossible interventions. Before describing our models, I will review some existing mathematical models of body weight dynamics.

## I.2 Mathematical models of body weight dynamics

In this thesis, I will focus only on models describing the evolution of body weight and body composition, due to their importance in health and disease. These models describe dynamics at long time scales, in the order of days to months. Nutritional systems can be modeled at different scales from intracellular process to organisms, including regulatory networks, signaling pathways, cellular growth and physiological processes. Such models take into account complex processes, with an important number of interacting components and non-linearities [de Graaf et al., 2009]. These models will constitute a base on which will be developed the model of body weight and body composition regulation by hormones presented in Chapter II.

Mathematical models of body weight dynamics that have been proposed over the past 25 years mostly concern humans and sometimes rodents. Human models provide more useful information in terms of health and can be useful for weight management. However, it is quite difficult, for ethical reasons, to perform overfeeding or underfeeding experiments in healthy humans. Models however can help determining relevant information that is not directly available from the experiments. These models are mainly used to study the effect of body weight change on body composition, the amount of caloric restriction needed to lose and maintain body weight, macronutrients usage or the effect of diet perturbations. As detailled in Section I.1, different diseases are linked to perturbations in the regulation of body weight and can be modeled, such as obesity. Most of them do not consider the regulation of food intake, which is only an input of the system. Models are then used to study the impact of changes in food intake on the dynamics of body weight and/or body composition. They can also be used to estimate the effect or the adherence to a weight loss program [Chow and Hall, 2014; Thomas et al., 2010b], however it is sometimes difficult to estimate initial conditions, such as basal energy expenditure or food consumption, for individuals leading to some uncertainties in the predictions [Brady and Hall, 2014].

In this section, I will present some mathematical models describing the regulation of body weight in humans or rodents, considering different biological assumptions, such as energy balance, macronutrient dynamics or adaptation of energy expenditure. First I will focus on macroscopic models, describing only the regulation of body weight, without details on body composition. Second, I will focus on modeling the regulation of body composition, based on energy balance and/or macronutrients dynamics. Then I will present a model of the hormonal regulation of body weight by leptin, which is the only model, to my knowledge, to consider leptin and thus the closest model to this thesis work.

Before presenting the models, I need to present the Minnesota semi-starvation experiment, which is often used to estimate parameter values or test the accuracy of the predictions of human models. This experiment was conducted at the end of World War II by Ancel Keys at the University of Minnesota. Its purpose was to study the physiological and psychological effects of starvation on humans and to determine the best refeeding strategy, in order to assist the victims of famine due to war [Kalm and Semba, 2005]. One of the main findings is that starvation affects personality as well as health.

This experiment was conducted on 36 healthy young men, who were conscientious objectors. They were submitted to different diets as follows:

1. **3 months** standardized period: $\sim$ 3200 kcal/day,
2. **6 months** semi-starvation: $\sim$ 1800 kcal/day, in the form of foods consumed in Europe during the war (for example potatoes and turnips),
3. **3 months** refeeding, with 4 different diets.

During this time, they were expected to perform various activities to keep an energy expenditure close to 3000 kcal/day. Biometric measurements were collected and the energetic content of the diet was individually adapted each week in order to lose 25% body weight by the end of the period of starvation [Kalm and Semba, 2005].

### I.2.1 Macroscopic models

In this section, I present models considering only the dynamics of body weight without considering body composition. These models are simple but allow to take into account phenomena widely ignored in modeling such as day to day stochasticity in energy intake and expenditure or psychological feedback on food intake to limit body weight gain.

In [Horgan, 2011], a simple discrete model of body weight dynamics in humans is presented to study different patterns of body weight change. This model only takes into account body weight description, without distinctions in body weight composition. Body weight $W$ at time $i+1$ is defined as $W_{i+1} = W_i + C(I_i - E_i)$, with $E_i$ the energy expenditure at time $i$, $I_i$ the energy intake and $C$ the cost of body weight change (see Figure I.8). Energy intake is normally distributed around a mean value $\mu_I$ and energy expenditure is normally distributed around a function of body weight. If energy intake is considered to be equal to its mean value, this model predicts a set-point for body weight. Body weight will then fluctuate around the set-point if the mean energy intake does not change. A decrease or increase in energy intake will induce a change in the set-point. This model includes

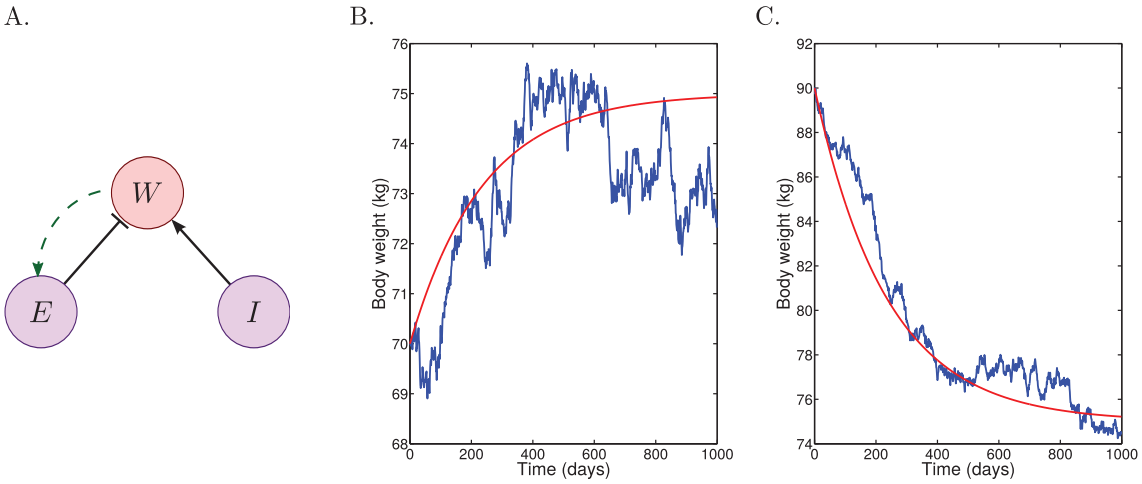


**Figure I.8** – Representation and results of [Horgan, 2011] model. A. Schematic representation of the model. $I$ and $E$ are random values normally distributed. The dashed arrow indicates the influence of body weight on energy expenditure. B. Example of a realisation of the model (in blue) compared with the approximation for a constant intake (in red), with a set-point equal to 75 kg, starting from 70kg. C. Example of a realisation of the model (in blue) compared with the approximation for a constant intake (in red), with a set-point equal to 75 kg, starting from 90kg.

the stochasticity in daily food consumption, which is often observed, due to conscious or unconscious regulations. The impact of these daily perturbations around the mean is limited at long time scales, yet the body weight depends on the mean energy intake. This result is also obtained by other models [Chow and Hall, 2014].

There exist other models considering a body weight set-point, such as [Kozusko, 2001] and [Tam et al., 2009]. In [Kozusko, 2001], a model predicting the evolution of body weight is defined as follows: $k\ dW/dt = C - E$, with $k$ the conversion of energy to body weight change, $C$ the caloric intake, $E$ the expended energy and $W$ the body weight (see Figure I.9 A). This model considers set-points for body weight, energy expenditure and fat content of the body and metabolic adaptations in response to weight loss based on these set-points. Energy expenditure is estimated by considering the current body weight and the value of the set-points. Results are compared to previously published formulas to predict energy expenditure, based on their ability to reproduce the evolution of body weight observed in the Minnesota semi-starvation experiment, knowing the caloric intake. It is then possible to determine the caloric requirement to maintain a body weight lower than the set-point [Kozusko, 2001].

In [Goldbeter, 2006], an ODE based model of body weight dynamics is presented. This model takes into account a psychological regulation of energy intake which induces a reduction in energy intake once a threshold body weight is reached (see Figure I.9 B for a

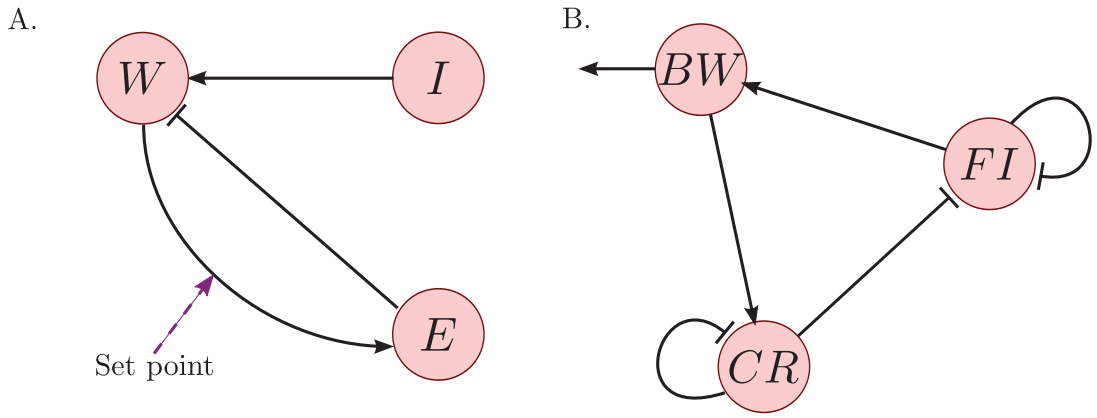


**Figure I.9** – Schematic representation of [Kozusko, 2001] and [Goldbeter, 2006] models. A. Representation of [Kozusko, 2001] model. Body weight $W$ increases with intake $I$ and decreases with energy expenditure $E$. Energy expenditure depends on the body weight and the body weight set-point. B. Simplified schematic representation of the interacting components in [Goldbeter, 2006] model. $BW$ stands for body weight, $FI$ for food intake and $CR$ for the cognitive restraint, which induces a downregulation of food intake when body weight reaches a certain threshold.

schematic representation). The psychological constraint then decreases with body weight, allowing for an increase in food intake which results in an increased body weight. Thus, this system displays oscillations in body weight, for some sets of parameter values, that could correspond to a consequence of "yo-yo" dieting. Over time or due to environmental perturbations, some parameters of the model could change, potentially inducing changes in the dynamics, such as a constant increase in body weight instead of oscillations. It should then be possible to stop body weight cycling by performing interventions on the system [Goldbeter, 2006].

Despite their apparent simplicity, these models can provide important insight on body weight regulation and reproduce observed mechanisms. However, more complexity in the models can bring additional information, for example introducing body composition, in particular fat mass which has an important impact on health.

### I.2.2 Modeling body composition dynamics

Modeling the evolution of body weight while considering changes in body composition brings important information for the management of weight changes in case of weight-related diseases, as fat mass and fat-free mass have different impacts on health. Moreover, as we have seen earlier, leptin is produced by adipocytes proportionally to fat mass. These

models can bring helpful insights for body weight regulation, for example the effect of macronutrients in diet on body composition, the adaptation occurring during a state of energy imbalance or the effect of a change in diet on obese or lean individuals [Hall, 2012].

The basic relationship described by these models corresponds to the linked evolution of fat and lean mass in the body. Gain and loss of body weight imply changes in both fat and lean mass. These models have practical applications in public health, to predict evolution of fat mass and fat-free mass during weight loss (in obesity) or weight regain (in cachexia or anorexia nervosa) or help designing a sustained weight loss program (for example the body weight simulator provides a practical tool to estimate the effect of caloric restriction and increased physical activity on weight loss and weight maintenance [Hall et al., 2011]).

These models are fitted and compared to experimental data corresponding to overfeeding or caloric restriction in humans or in rodents. Applications of these models range from growth [Hall et al., 2013], weight loss [Hall and Baracos, 2008; Song and Thomas, 2007; Thomas et al., 2011] and weight gain due to overfeeding [Chow and Hall, 2008; Hall, 2006] or pregnancy [Thomas et al., 2012] in humans to the description of rodents body weight evolution [Guo and Hall, 2009, 2011; Tam et al., 2009]. Predictions of these models are much more accurate than the assertion that a 3500 kcal reduction in food intake leads to the loss of 1 lb, mainly due to the loss of body fat [Hall, 2008; Thomas et al., 2014]. A sustained reduction in food intake over time does not induce a constant reduction in body weight, the decrease is important during the first weeks of the caloric restriction before slowing and eventually stopping. The percentage of body weight loss imputable to fat mass depends on the initial body composition and on the caloric restriction. Some models also take into account the adaptation of energy expenditure, due to changes in diet induced thermogenesis, metabolic rate and adaptive thermogenesis, occurring during weight loss [Hall, 2012], as will do the model from Chapter II [Jacquier et al., 2014].

In this section, a few representative models of the dynamics of body weight composition are presented. The work presented in Chapters II, III and IV is based on some of these models [Guo and Hall, 2009, 2011; Tam et al., 2009]. They are based on the description of different aspects of body composition regulation, in particular energy balance and macronutrient dynamics and their impact on body composition.

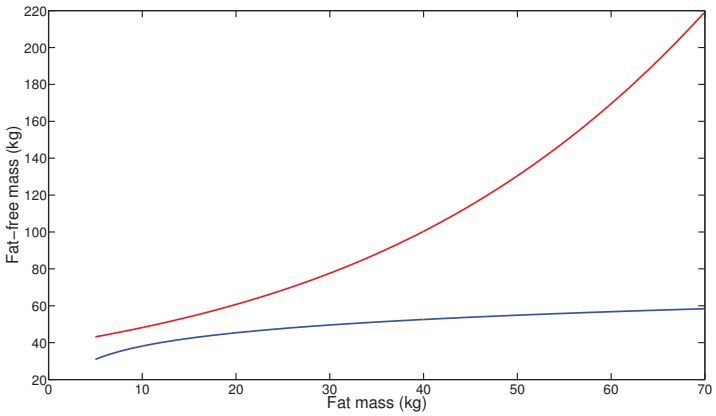


**Figure I.10** – Illustration and comparison of the models from [Forbes, 1987] and [Thomas et al., 2010a]. The evolution of fat-free mass is predicted as a function of fat mass, for a woman, for Equations (I.1) (in blue) and (I.3) (in red). For the equation from [Thomas et al., 2010a], age is fixed at 30 and height at 160 cm. For low fat mass, the predicted fat-free mass is close for both formulas but they diverge for increasing fat mass.

### I.2.2.1 Forbes model

One of the first models describing the relationships between fat mass and fat-free mass was developed by Forbes in 1987 from experimental data in women [Forbes, 1987], as follows:

$$\frac{\mathrm{d}FFM}{\mathrm{d}FM} = \frac{10.4}{FM}, \tag{I.1}$$

with $FFM$ the lean body mass and $FM$ the fat mass (see Figure I.10). A similar formula was latter obtained for men. This model was used to predict the evolution of lean mass relatively to body weight during energy restriction and overfeeding.

This equation was latter modified in [Hall, 2007] to describe macroscopic changes in body weight, as Forbes equation is only valid for small weight changes. The change of body composition is defined as follows:

$$\frac{\Delta FFM}{\Delta BW} = 1 + \frac{FM_i}{\Delta BW} - \frac{10.4}{\Delta BW}\,\mathrm{W}\left(\frac{1}{10.4}\exp\left(\frac{\Delta BW}{10.4}\right)FM_i\exp\left(\frac{FM_i}{10.4}\right)\right), \tag{I.2}$$

with $BW$ the body weight, $FM_i$ the initial fat mass and W the Lambert W function [1]. Thus body composition change is mainly determined by initial fat mass and body weight variation (in particular important weight variations, see Figure I.11) [Hall, 2007]. This

1. The Lambert W function, also called the omega function, corresponds to the inverse of the function $f(x) = x\exp(x)$, thus $W(x) = f^{-1}(x\exp(x))$.

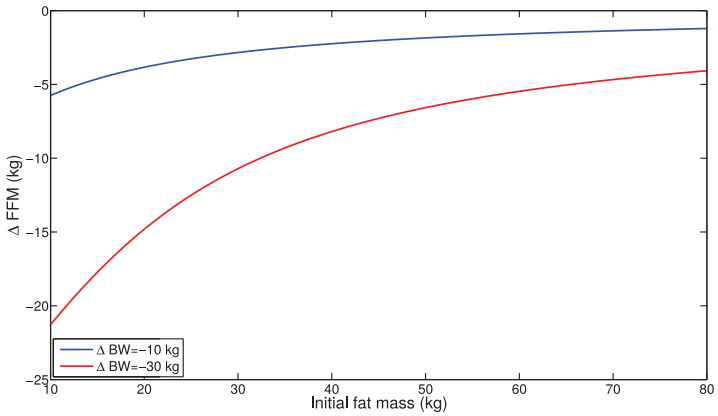


**Figure I.11** – Illustration of models from [Hall, 2007]. Change in fat-free mass is predicted as a function of the initial fat mass, by Equation (I.2), for different changes in body weight: -10 kg in blue and -30kg in red. The change in fat-free mass for a given reduction in body weight is then dependent on the initial fat mass, if fat mass increases, the change in fat-free mass decreases without reaching 0.

model can be used to determine the energy deficit necessary to lose a certain amount of body weight. For obese subjects, the above-mentioned assertion that a 3500 kcal reduction in food intake leads to the loss of 1 lb is close to the prediction, which is not the case for subjects with lower initial fat mass. However this model does not take into account adaptation of energy expenditure occurring during weight loss [Hall, 2008].

Another more precise expression of fat-free mass as a function of fat mass can be found in [Thomas et al., 2010a]. Fat-free mass $FFM$ is defined as a polynomial function of fat mass $FM$ (distinct for male and female) with dependence on age and height (see Figure I.10). For example, the equation for male has the following expression:

$$FFM(t) = e(H,A) + f(H,A)FM(t) + g(H,A)FM(t)^2 + h(H)FM(t)^3 - lFM(t)^4, \quad \text{(I.3)}$$

with $e$ to $h$ functions of the form $a+bH+cA$, $A$ the age and $H$ the height. These equations have been statistically determined from body composition experimental measurements. During weight loss, body composition follows the estimated variation from the model, except for some subjects with obesity surgery [Thomas et al., 2010a].

These models are only descriptive, however they can quite accurately predict the evolution of body composition during weight loss. In order to quantitatively predict the evolution of body weight and body composition, more biological informations are needed, such as energy intake and energy expenditure, as well as mechanisms regulating them.

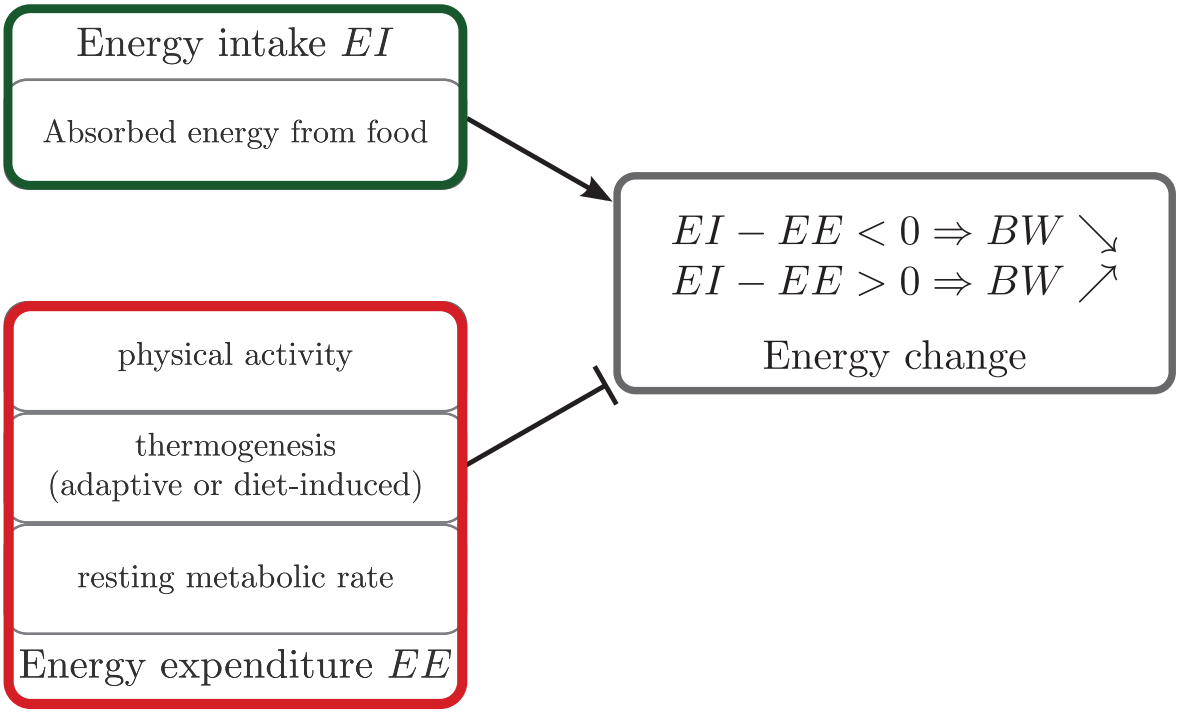


**Figure I.12 –** Energy change, also called energy balance, is equal to the difference between energy intake and energy expenditure and impacts the variation of body weight: if energy change is positive there will be storage of energy in the form of glycogen, protein or triglycerides and if energy change is negative this storage will be depleted.

### I.2.2.2 Models of energy balance

Models of energy balance are based on the application of the first law of thermodynamics, which states the conservation of energy, on the body which is considered as an open system with input in the form of food consumed [Thomas et al., 2009]. Energy intake corresponds to the food consumed while energy expenditure is divided into different components, including resting metabolic rate, physical activity or thermogenesis [Thomas et al., 2009] (see Figure I.12). It is assumed that there is a cost in energy to store energy in the form of glycogen, triglyceride or protein, in particular for positive energy balance [Hall, 2010a].

In [Alpert, 1979], a two-compartments model of body weight, based on energy conservation is developed. The model takes into account variations of the fat store $f$ and the fat-free store $l$, defined as the difference between body weight and fat mass, as follows:

$$\alpha \frac{\mathrm{d}f}{\mathrm{d}t} + \beta \frac{\mathrm{d}l}{\mathrm{d}t} = I - E, \tag{I.4}$$

with $\alpha$ and $\beta$ the energy conversion factors relative to fat and fat-free stores, $I$ corre-

sponding to the usable energy intake and $E$ the energy expenditure, decomposed into basal metabolic rate, wasted energy and physical activity. From Equation (I.4), differential equations for fat and fat-free stores can be deduced. This model is then applied to data from the Minnesota semi-starvation experiment to test the relevance of the assumptions. The prediction of the evolution of body weight during semi-starvation is correct despite some inaccuracies, imputed by the authors to technical limitations [Alpert, 1979].

In [Song and Thomas, 2007], a mathematical model of body composition during starvation, based on the first law of thermodynamics, is presented. This model describes the dynamics of fat mass, muscle mass and ketone bodies (see Figure I.13). Ketone bodies are molecules produced by the liver from fatty acids during caloric restriction and are used as alternatives to glucose by the brain [Song and Thomas, 2007]. In normal food conditions, there is a negligible production of ketone bodies. Energy expenditure is divided into 4 components: diet induced thermogenesis, physical activity, adaptive thermogenesis and basal metabolic rate. As this model is applied during starvation, energy intake is assumed to be equal to 0, as well as diet induced thermogenesis and physical activity. Adaptive thermogenesis is also assumed to be equal to 0, indicating a constant environment. Energy expenditure is then equal to the basal metabolic rate, defined by a function of fat mass $F$ and fat-free mass (equal to the sum of muscle mass $M$ and a constant mass $L_0$ representing organs and bones): $C + \kappa(L_0 + M + F)$. The model is defined as follows:

$$\begin{cases} \dfrac{\mathrm{d}F}{\mathrm{d}t} &= -r(K)F - \dfrac{1}{\lambda_F}\dfrac{F}{F+M}(C + \kappa(L_0 + M + F)), \\ \dfrac{\mathrm{d}M}{\mathrm{d}t} &= -\dfrac{1}{\lambda_M}\dfrac{M}{F+M}(C + \kappa(L_0 + M + F)), \\ \dfrac{\mathrm{d}K}{\mathrm{d}t} &= Vr(K)F - b, \end{cases} \tag{I.5}$$

with $K$ the ketone bodies, $\lambda_F$ and $\lambda_M$ the caloric content of stored fat and protein, $r(K)$ the conversion of fat mass into ketone bodies, $V$ the conversion constant of fat mass into ketone bodies and $b$ the ketone usage by the brain. This model allows to estimate the survival time under starvation, assuming that death occurs when ketone bodies are depleted or when fat-free mass reaches half of its initial value. The survival time is higher for an obese individual (approximately 90 days) than for a normal one (approximately 50 days), due to a more important initial fat mass [Song and Thomas, 2007].

Starvation, presented in the previous model, is an extreme case of caloric restriction. Controlled caloric restriction is more common, in particular it is used to induce weight loss. In [Hall and Jordan, 2008], a model of the change in body weight and body composition

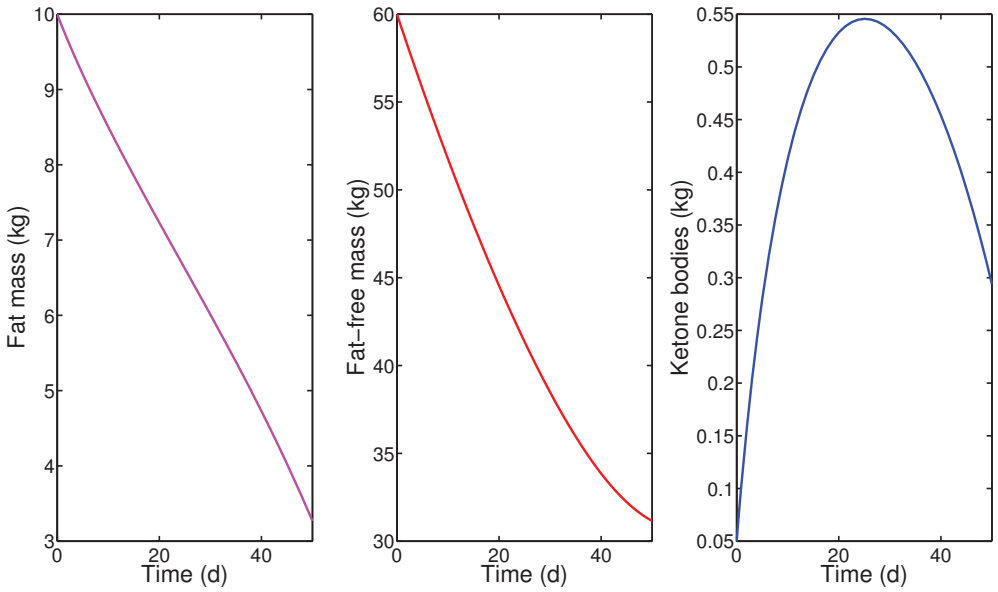


**Figure I.13** – Illustration of the model from [Song and Thomas, 2007], in the case of starvation: evolution of fat mass, fat-free mass and ketone bodies during starvation, as described by Equation (I.5). Parameter values are taken from[Song and Thomas, 2007], initial conditions correspond to a non-obese individual and the duration of the simulation corresponds approximately to the survival for a non-obese individual [Song and Thomas, 2007]. As expected from the starvation state, fat mass and fat-free mass decrease over time while ketone bodies increase during the first 25 days before decreasing.

is presented and provides a practical tool to estimate the changes in intake and physical activity necessary to maintain a reduced body weight and avoid weight regain. In this model, energy expenditure (see Equation (I.6)) at equilibrium is defined as a function of intake $EI$(for diet induced thermogenesis), body weight (for physical activity) and body composition:

$$EE = K + \beta EI + \gamma_{FFM} FFM + \gamma_{FM} FM + \delta(FFM + FM), \tag{I.6}$$

with $FFM$ the fat-free mass, $FM$ the fat mass, $\delta$ the energy cost of physical activity, $\beta$ the thermic effect of feeding, $\gamma_{FM}$ and $\gamma_{FFM}$ representing the contribution of fat mass and fat-free mass to the resting metabolic rate. Knowing that, at a maintained body weight, energy intake equals energy expenditure and assuming that the change in body composition follows the equation from [Hall, 2007], it is possible to estimate the change in body weight as a function of the change in energy intake, as well as the change in physical activity and initial body weight and composition. It is then possible to calculate the change in fat-free mass from the change in body weight or to estimate the changes in energy intake and physical activity needed to maintain a reduced body weight. The

model is able to accurately predict the change in body weight resulting from changes in energy intake and physical activity, based on experimental data. The same reduction in intake can lead to different changes in body weight, depending on the initial fat mass: the decrease is more important for high initial fat mass [Hall and Jordan, 2008].

It is well known that some adaptations in energy expenditure occur during body weight change, such as adaptive thermogenesis. In [Thomas et al., 2009], the authors present a mathematical model describing the evolution of body composition with adaptations in resting metabolic rate due to body weight change and age, and in non-exercise activity thermogenesis (see Equation (I.7)). In this model, fat-free mass is assumed to be a function of fat mass, linearized from Forbes model. Similarly to most models, energy intake is an input of the system and is not estimated. Energy expenditure $EE$ is divided into four components:

- diet induced thermogenesis ($DIT$), which is proportional to energy intake $EI$,
- physical activity ($PA$), which is proportional to body weight,
- resting metabolic rate ($RMR$), depending on age and adapting to caloric change as follows: $RMR = (1-a)(a_i W^{p_i} - \gamma_i A)$, with $a$ the percentage of metabolic adaptation, $A$ a time varying function representing age, $\gamma_i$ the dependance on age, $W$ the body weight (sum of fat mass and fat-free mass), $a_i$ and $p_i$ the parameters relative to body weight in the Livingston-Kohlstadt formula predicting resting metabolic rate [Livingston and Kohlstadt, 2005],
- non-exercise activity thermogenesis ($NEAT$), whose change is proportional to the change in energy expenditure ($\Delta NEAT = r\Delta EE$), leading to

$$NEAT = r/(1-r)(DIT + PA + RMR) + c$$

.

Simplifying all these components leads to the equation for fat mass, as follows:

$$\frac{\mathrm{d}F}{\mathrm{d}t} = \frac{EI - EE}{\lambda} = \gamma(t) - \eta F - \delta(mF + b)^{p_i}, \tag{I.7}$$

with $\lambda$ representing the relationship between fat mass and fat-free mass and $\gamma(t)$ a function depending on energy intake. This model is then compared to experimental data for overfeeding and underfeeding and is able to predict body weight after some time more accurately, giving better results than a model without non-exercise activity thermogenesis [Thomas et al., 2009].

In [Thomas et al., 2011], a mathematical model describing the evolution of body compo-

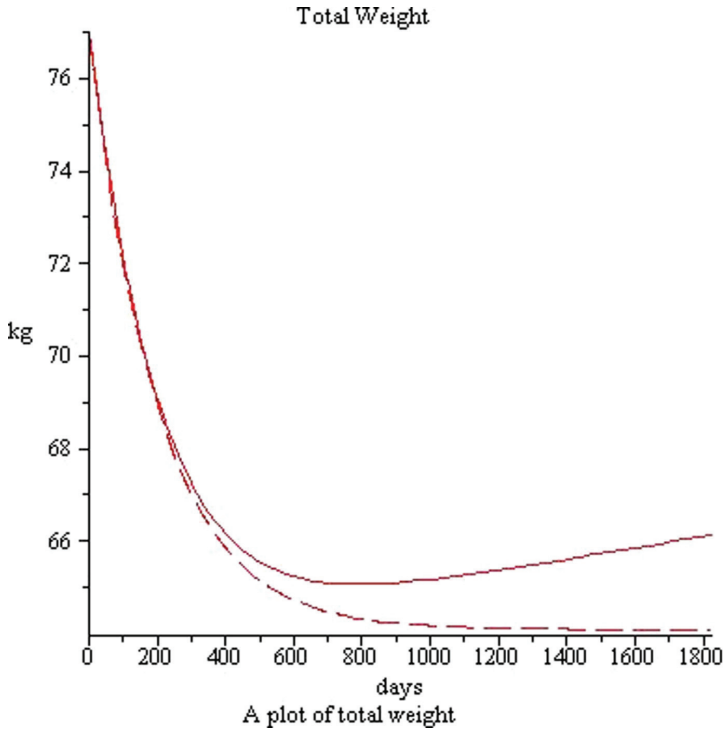


**Figure I.14** – Example of a predicted temporal evolution of body weight (from [Thomas et al., 2011]), with an age considered constant (dashed line) or changing with time (solid line). Initial condition are 77 kg (body weight), 172 cm (height) and 44 years (age), with a caloric intake of 2200 kcal/day.

sition, based on the previous model (I.7) [Thomas et al., 2009] and the algebraic Equation (I.3) from [Thomas et al., 2010a], is presented. This model is able to predict individual weight change during underfeeding or overfeeding and can also be used to estimate the adherence to a diet by comparing predicted and observed evolutions of body weight (see Figure I.14). The only inputs of this model are age, height, initial weight and gender. Energy intake is considered to be non-constant in this model and parameter values are estimated from the literature and from experimental data on weight stable subjects or during weight change. Compared to the previous model (Equation (I.7)), this model gives better predictions for the final body weight [Thomas et al., 2011]. This model can be used to estimate energy intake during weight loss from periodic body weight data and can help estimate the adherence to the diet and its impacts [Thomas et al., 2010b].

The above-mentioned models describe the regulation of body composition, based on the description of energy intake and energy expenditure. These models are descriptive based on data fitting, and are then only valid for specific populations. Macronutrients (glycogen, protein and fat) from food are used as energy by the organism, and their dynamics regulate the separation of the body into fat mass and fat-free mass depending on the composition of the diet. However, the models in this section do not describe explicitly the evolution

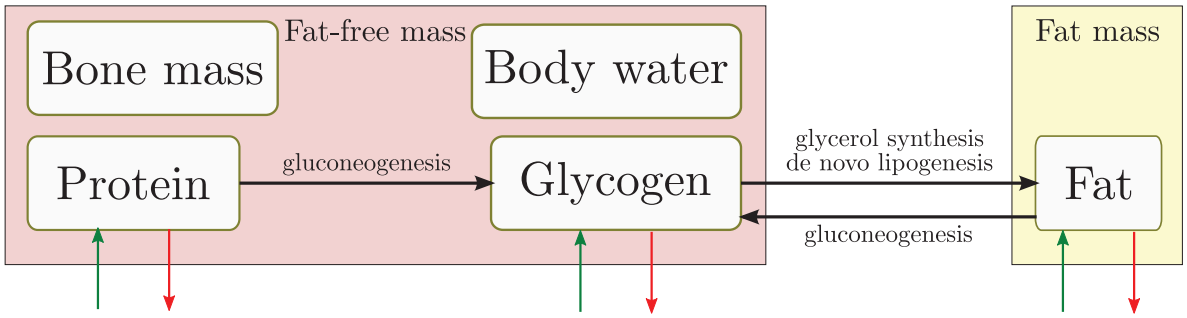


**Figure I.15** – Schematic representation of the macronutrients balance model with the distinction of fat mass and fat-free mass from [Hall, 2006]. Green arrows indicate macronutrient inputs (carbohydrates, protein and fat) while red arrows indicate oxidations.

of macronutrients, even if they sometimes use their interactions to develop the model. In the next section I will present models based on the description of macronutrients to study the dynamics of body weight and composition.

### I.2.2.3 Macronutrients dynamics

Macronutrients correspond to protein, fat and carbohydrates and are consumed in important quantities by the body, contrary to micronutrients (vitamins, minerals). Macronutrients are obtained from food with various absorption rates. They can be stored or directly used to obtain energy, by oxidizing them to carbon dioxide and water.

Some models describe the dynamics of macronutrients in the body: protein, fat and glycogen, at long time scales [Hall, 2006, 2012]. These macronutrients are obtained from the consumed food and complex mechanisms regulate their utilization (and storage), despite changes in the composition of the food. Fat-free mass variations correspond to the variations in protein and glycogen contents, as well as bone mass and body water. Some fluxes exist between these macronutrients, such as gluconeogenesis leading to the conversion of protein or fat to glycogen or de novo lipogenesis leading to the creation of fat from glycogen (see Figure I.15). The rates of these fluxes are influenced by protein, glycogen and fat contents in the body [Hall, 2006].

Modeling these macronutrients dynamics allows to determine the oxidation rates of the different macronutrients and the impact of food composition on body composition. Variations in caloric intake and food composition induce adaptations in oxidation and conversion rates of macronutrients [Hall, 2006] (see Figure I.15). The model from [Hall, 2006] is able to reproduce observed evolutions of body weight and fat mass of the Minnesota semi-

starvation experiment, including the overshoot of fat mass observed during refeeding and to predict energy expenditure and metabolic fluxes, knowing the daily food consumption and composition [Hall, 2006]. It is used to investigate the effects and potential treatments of cancer cachexia [Hall and Baracos, 2008]. Cancer cachexia is associated with changes in metabolism and reduced food intake, however it is difficult to measure energy expenditure and metabolic fluxes in these patients due to their condition. An increase in lipolysis, proteolysis, glycolysis and gluconeogenesis, due to cancer cachexia, are included in the model, as well as the addition of a tumor with a specific metabolic rate [Hall and Baracos, 2008].

Combining a simplified version of this model with Forbes equations on body composition (Equation (I.1)) allows to take into account adaptations in substrate utilization to the diet and exercise, and their impact on body composition [Hall et al., 2007]. Changes in the macronutrient composition of the diet can impact the oxidation rates, in particular changes in protein and carbohydrate (but not fat), induce adaptation of oxidation rates. This allows to predict changes in body composition and macronutrient utilization, knowing energy intake and energy expenditure for under- and overfeeding [Hall et al., 2007]. Experimental data allow to complexify the model and apply it to different biological questions, as in [Jordan and Hall, 2008] where a model of body composition, energy intake and expenditure and macronutrient during infant growth is presented, based on [Hall et al., 2007] and experimental data. Knowing the evolution of body weight, body composition, fat content in the diet and carbon dioxide production during the first two years of life, the model is able to predict energy intake and expenditure and oxidation rates during growth [Jordan and Hall, 2008].

In [Chow and Hall, 2008], the authors provide a general model of the long-term dynamics of body weight and body composition based on macronutrient flux balance. Changes in body weight depend on the energy content of the diet, its composition in carbohydrate, fat and protein and on energy expenditure. Positive energy balance results in the storage of energy in the form of fat $F$ and in the form of protein $P$ and glycogen $G$ in lean tissue. Macronutrient dynamics is described as follows:

$$\begin{cases} \rho_F \dfrac{\mathrm{d}F}{\mathrm{d}t} &= I_F - f_F E, \\ \rho_G \dfrac{\mathrm{d}G}{\mathrm{d}t} &= I_C - f_C E, \\ \rho_P \dfrac{\mathrm{d}P}{\mathrm{d}t} &= I_P - (1 - f_C - f_F) E, \end{cases} \tag{I.8}$$

with $\rho_F$, $\rho_C$ and $\rho_P$ the energy densities of fat, glycogen and protein, $I_F$, $I_C$ and $I_P$ the intakes of fat, carbohydrate and protein, $E$ the expended energy and $f_F$ and $f_G$ the fractions of energy due to the combustion of fat and glycogen. The model (I.8) can be reduced to a two-compartments model of fat and lean mass, equal to the sum of protein, glycogen and a constant accounting for bone mass. Due to the limited storage of glycogen in the body, $dG/dt$ is assumed to be equal to 0 and lean mass variations depend only on protein, as follows:

$$\begin{cases} \rho_F \dfrac{\mathrm{d}F}{\mathrm{d}t} &= I_F - fE, \\ \rho_L \dfrac{\mathrm{d}L}{\mathrm{d}t} &= (I_C + I_P) - (1-f)E. \end{cases} \tag{I.9}$$

This model can be simplified into an energy partition model [Hall, 2012] by assuming that body composition trajectories follow a prescribed path (similarly to Forbes theory), with the definition of an energy partition function. The model is defined as follows:

$$\begin{cases} \rho_F \dfrac{\mathrm{d}F}{\mathrm{d}t} &= (1-p)(I-E), \\ \rho_L \dfrac{\mathrm{d}L}{\mathrm{d}t} &= p(I-E), \end{cases} \tag{I.10}$$

with $p$ a function defining the fraction of energy stored as lean tissue. Models (I.8) to (I.10) can either display fixed points or invariant manifolds. Most models of body weight dynamics can be reduced to one of these three models [Chow and Hall, 2008].

In 2010, Hall proposed a mathematical model, based on [Hall, 2006], combining macronutrient balance, body composition and adaptation of energy expenditure [Hall, 2010b]. This model allows to predict the effect of diet perturbations on fuel selection (see Figure I.16) and energy expenditure, which adapts to the diet resulting in changes in body weigh and body composition, and takes into account the dynamics of fluids (intracellular and extracellular). Energy is assumed to be conserved, with energy intake corresponding to the sum of macronutrients energy (carbohydrates, proteins and fats) and energy expenditure divided into the following components:

- thermic effect of feeding, depending on food composition,
- physical activity, depending on body weight and adaptive thermogenesis (depending on intake),
- resting metabolic rate which includes the energy required for metabolic fluxes.

Imbalances between intakes and utilization of macronutrients result in body composition changes. Fat mass depends on fat intake, de novo lipogenesis, ketone production and fat oxidation. Fat-free mass is divided into multiple components, including glycogen,

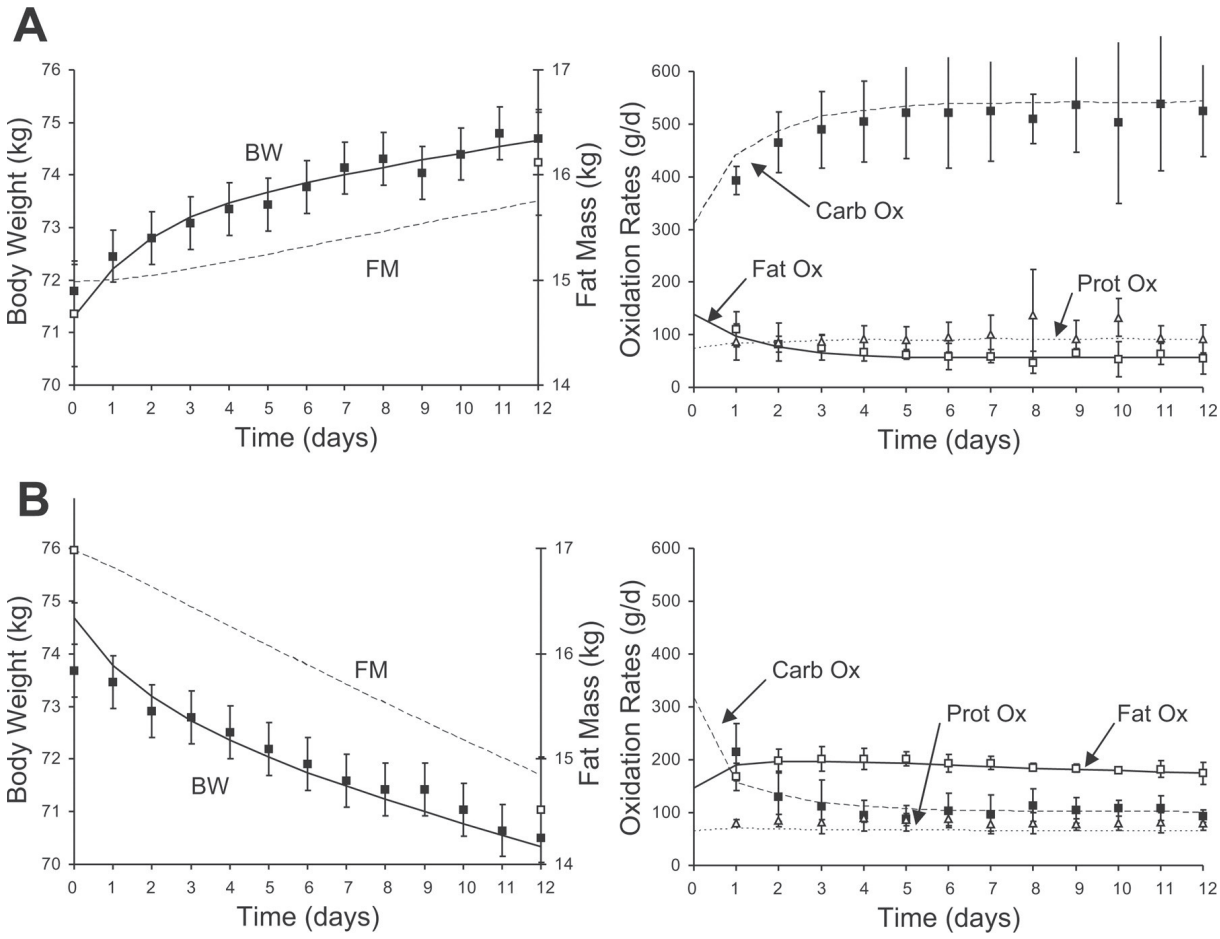


**Figure I.16** – Comparison between predicted and observed evolutions of body weight and macronutrients oxidation rates during overfeeding (top) and underfeeding (bottom) (from [Hall, 2010b]).

protein, bone mass and extracellular water, which depends on the change in sodium in the diet. This complex model is calibrated using human experiments and the predictions are consistent with other feeding studies. This model is valid for both obese and non-obese individuals and the only input is the food intake during the time-course of the experiment. Knowing the evolution of body weight, it is also possible to estimate energy intake [Hall, 2010b].

Along with these models in humans, Guo and Hall proposed a mathematical model of energy metabolism in mouse [Guo and Hall, 2009, 2011]. This model takes into account fat mass, fat-free mass, energy expenditure and metabolic fuel selection, and is based on the first law of thermodynamics (law of energy conservation). Similarly to Forbes body composition function for humans [Forbes, 1987], a relationship between fat mass and fat-free mass variations is derived from the experimental data, as follows:

$$\frac{\mathrm{d}FFM}{\mathrm{d}FM} = c + d\exp(kFM). \tag{I.11}$$

Energy expenditure is divided in multiple components, including thermogenesis, physical activity, basal metabolic rate and thermic effect of feeding. Energy expenditure is then a function depending on environmental conditions, fat mass, fat-free mass (and their variations) and energy intake. To consider fuel selection, macronutrients (carbohydrates, fat and protein) are taken into account (similarly to the relationship between macronutrients described in Figure I.15). Macronutrients are brought by food, can be stored, oxidated or converted into other nutrients. Combining the equations describing the evolution of macronutrients with the equations for fat mass and fat-free mass allows to evaluate the respiratory quotient, depending on the oxidation rates and its dynamics during diet induced changes in body weight. Calibration of the model is performed using experimental data on mice submitted to normal or high-fat diet, with switches between diets, to account for weight gain and loss. The model is then able to reproduce the evolutions of body weight observed in the experiments.

The models described in this section are based on the description of macronutrient dynamics in order to describe body composition. Hormones, in addition to their effect on food intake and energy expenditure, participate in the regulation of macronutrients dynamics, for example leptin induces lipolysis in white adipose tissue, but they are not explicitly considered in the models.

### I.2.3 Hormonal regulation of body weight

Previously described models do not explicitly take into account hormonal regulators of food intake and energy expenditure, even if they model phenomena regulated by hormones. Models accounting for the effects of hormones on body weight are not common, despite increasing knowledge on their actions.

In [Tam et al., 2009], a physiologically-based mathematical model of energy homeostasis regulated by leptin in mice is proposed (see Figure I.17). Leptin regulation induces compensations in energy intake and energy expenditure to limit changes in body weight. The authors study the effect of perturbations of the system, such as a disruption of the leptin regulatory pathway, characterizing leptin resistance, or changes in the caloric content of the food. This model is based on ordinary-differential equations, describing the dynamics

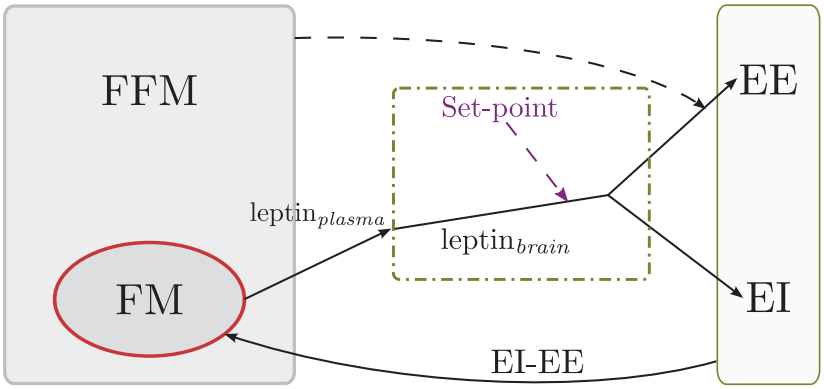


**Figure I.17 –** Schematic representation of the model of leptin regulation of body weight in mice from [Tam et al., 2009]. Fat mass (FM) produces leptin in plasma, which enters the brain. Brain leptin impacts energy intake (EI) and energy expenditure (EE). Body weight (fat mass and fat-free mass) is involved in the regulation of energy expenditure (dashed arrow). Fat-free mass is considered constant, but fat mass changes depending on the energy balance (EI-EE). In the case of a set-point, the regulation of energy intake and energy expenditure by brain leptin is modified relatively to this set-point. In the case of leptin resistance, brain leptin is reduced.

of plasma leptin $L_{\text{plasma}}$, brain leptin $L_{\text{brain}}$ and energy balance $E$, as follows,

$$\begin{cases} \dfrac{\mathrm{d}(L_{\text{plasma}}BV)}{\mathrm{d}t} &= R_{\text{syn}}\dfrac{E}{\rho_F} - CL_{\text{plasma}}, \\ L_{\text{brain}} &= \dfrac{k_1 L_{\text{plasma}}}{k_2 + L_{\text{plasma}}} + k_3 L_{\text{plasma}}, \\ \dfrac{\mathrm{d}E}{\mathrm{d}t} &= \rho FI - k_6\left(\dfrac{E}{\rho_F} + FFM\right)\left(1 + \dfrac{k_7 L_{\text{brain}}}{k_8 + L_{\text{brain}}}\right), \end{cases} \tag{I.12}$$

with $BV$ the blood volume, $R_{\text{syn}}$ the rate of leptin synthesis, $C$ the elimination rate for leptin, $\rho$ the energetic density of the food, $\rho_F$ the energetic density of fat and $FFM$ the fat-free mass, which is assumed to be constant. Body weight is then calculated from fat mass, defined as $E/\rho_F$, and fat-free mass.

This model can account for an explicit set-point or a settling-point, by modifying the equation describing the regulation of food intake $FI$. When considering a settling-point, food intake is defined as

$$FI = k_4\left(1 - \frac{L_{\text{brain}}}{k_5 + L_{\text{brain}}}\right), \tag{I.13}$$

while the equation describing food intake in the case of a set-point is

$$FI = a_1(L_{\text{brain}} - SP) + a_2\int_0^t (L_{\text{brain}} - SP)\mathrm{d}t + c_1, \tag{I.14}$$

with $SP$ the set-point. In both Equations (I.13) and (I.14), food intake is regulated by leptin. Energy expenditure is also regulated by leptin and by body weight (see System (I.12)). At the time-scale considered (days to weeks), only fat mass is changing, depending on the difference between energy intake and energy expenditure, but fat-free mass is assumed to remain constant. Leptin resistance is modeled via a reduction in leptin transport to the brain, resulting in a system with multiple steady-states. The model is able to reproduce experimental evolutions of body weight in mutant mice lacking leptin. In the settling-point model, body weight does not change a lot, despite changes in the energetic density of the food which are not able to model the development of obesity.

The settling-point model, with a body weight determined by the evolution of all variables, gives results more consistent than the set-point model, which tends to match a determined body weight, to model diet-induced obesity. A combination of both models is more appropriate in the case of starvation, as well as diet-induced obesity.

In this section, I presented some models of the regulation of body weight, based on multiple assumptions. Most models consider body composition, due to its importance for health, but some simplified models only consider body weight. One of the main assumptions is that body change is linked to energy balance, the difference between energy intake and energy expenditure. Energy expenditure can be considered only as a function of body weight or composition, or divided into multiple biologically relevant components, which may adapt, for example to food intake. However, only one of these models explicitly described the effect of leptin and none of them considered other hormones, despite their important role in the regulation and the complex interactions occurring between them. Another important assumption is to consider the dynamics of macronutrients, which are brought by food, as the base for the partition of body weight into fat and fat-free mass. Leptin is the main regulatory hormone, however ghrelin and insulin have also a non-negligible impact on the regulation of food intake and interact with leptin. These hormones will be considered in Chapters II and III, to propose models based on energy balance, to study the dynamics of food intake and body weight on the impact of caloric restriction on this evolution of body weight.

## I.3 Thesis work

I introduce here my thesis work, consisting in the development of models taking into account the hormonal regulation of food intake and body weight, and focusing in particular on the role of leptin, with two main applications: the effect of caloric restrictions on the dynamics of body weight and the development of leptin resistance.

In Chapter II, I introduce a model of the regulation of food intake and body weight in rats, which has been published in 2014 in PLoS One [Jacquier et al., 2014]. This mathematical model is based on a system of ordinary and delay differential equations, describing the evolution of body weight, body composition, food intake and energy expenditure. The regulation of food intake is based on hormonal regulations by leptin and ghrelin. This model is applied in the case of caloric restrictions and compared to experimental data. It allows to study the impact of different food patterns on the evolution of body weight in rats, experimentally and by simulations, and to evaluate the importance of adaptation in energy expenditure in the case of caloric restriction.

Experiments were conducted on Wistar rats, divided into different groups: one control group receiving *Ad libitum* feeding and three restricted groups receiving a total of 80 % of the normal diet, with different patterns of food distribution. The evolution of food consumption and body weight were monitored during the duration of the experiment. By the end of the experiment, significant differences appeared between the groups. As expected, body weight was significantly lower for the restricted groups compared to *Ad libitum* group. Differences in food consumption and body weight also exist between the restricted groups. In particular one of the restricted groups consumed less food but had a higher body weight. These results indicate that some adaptations occurred in energy expenditure, relatively to the amount of food consumed and the pattern of food intake.

The mathematical model takes into account fat mass, fat-free mass, hunger (defined as the amount of food consumed in the absence of restriction), plasma leptin, plasma ghrelin, plasma glucose, the rate of energy expenditure and the food availability. Leptin, ghrelin and glucose are known regulators of food intake, leptin and glucose leading to a decrease in food intake, while ghrelin induces food intake. Fat mass and fat-free mass are described by two coupled equations adapted from a previous model of body weight dynamics in mice [Guo and Hall, 2009, 2011]. The change in body weight depends on the difference between energy intake and energy expenditure. Energy intake depends on hunger and food available while energy expenditure is defined as a function of fat mass and fat-free

mass. The rate of energy expenditure is assumed to adapt to the previously consumed food, and is described by a delay differential equation. Leptin increases proportionally to fat mass, while ghrelin production is inhibited by food consumed. Hunger is regulated by leptin, ghrelin and glucose, relatively to the biological properties of these regulators of food intake.

In order to test the predictive power of the model, we used experimental data from the control group and one of the restricted groups to estimate parameter values. We then simulated the calibrated model for the remaining groups, with only the initial condition and food availability as inputs, to predict the evolution of body weight and food intake. We showed that our model is able to predict quite accurately the evolution of body weight and food intake observed experimentally, for the restricted groups and for the control group with *Ad libitum* food. In particular, we predicted leftover food in some groups as observed experimentally and that the pattern of food availability has an impact on body weight dynamics as well as the total food consumption. Assuming that food intake induces an adaptation in the rate of energy expenditure with a memory of food intake gives better results than considering a constant rate of energy expenditure, highlighting the importance of adaptations due to caloric restriction. Thus, due to food patterns and the delay in the adaptation of energy expenditure, we showed that one group can have a higher mean body weight than the others despite having a total food intake lower than the other groups.

In Chapter III, I present a model of the leptin mediated regulation of food intake and body weight, which has been published in 2015 in Mathematical Biosciences [Jacquier et al., 2015]. This model is based on a system of ordinary differential equations and takes into account leptin, leptin receptors and body composition. It allows to study the impact of perturbations, such as leptin injection or changes in food consumption, on the development of leptin resistance.

This model is based on the model presented in Chapter II and describes the dynamics of fat mass, food intake, leptin and leptin receptors. Fat-free mass is defined as a function of fat mass and initial conditions. Leptin is assumed to be a regulator of its receptors, which mediate the inhibition of food intake, in agreement with current biological knowledge. Changes in fat mass depend on the difference between energy in food intake and energy expenditure. Food intake is inhibited by the activation of leptin receptors by leptin, which is produced proportionally to fat mass. We assumed that leptin impacts both production and degradation of leptin receptors, with the condition that for high leptin concentration,

leptin receptors are downregulated.

This model displays between one and three positive equilibria, with a hysteresis. Two stable equilibria can coexist: one "healthy" equilibrium and one "obese/leptin resistant" equilibrium. The "healthy" equilibrium is characterized by low fat mass, low leptin and high number of leptin receptors while the "obese/leptin resistant" equilibrium has high fat mass, high leptin and a low number of receptors. Along with this analysis, some simulations were performed to study the impact of different perturbations on the dynamics of the model and the development of leptin resistance and obesity. We showed that progressive variations in the parameter determining the increase in food intake could lead to the development of leptin resistance. As the regulation of leptin receptors by leptin is reversible, it is possible to go back to the "healthy" equilibrium by opposite changes in parameter values. The impact of leptin on leptin resistance is also studied, by simulating the effect of constant leptin injection on the dynamics and comparing to experimental data from [Pal and Sahu, 2003]. The injection initially induces a decrease in food intake and body weight, followed by a return to the initial state due to the development of leptin resistance. The model allows to reproduce this dynamics, after estimation of parameter values.

In Chapter IV, a simplification of the models from Chapters II and III is presented, in order to avoid positivity issues arising from the previous models and simplify the analysis of the system, while keeping the properties of the models. I will then use this simplification to build a model combining regulatory mechanisms from both previous models: this model takes into account the regulation of food intake by leptin, ghrelin and glucose, adaptation of energy expenditure and the dynamics of leptin receptors, which are involved in leptin resistance.

The main idea is to replace the equations for fat mass and fat-free mass by a single equation describing body weight dynamics. As we also need to describe fat mass for leptin production, a function determining fat mass from body weight is developed, and fitted to experimental data. Energy expenditure is considered to be proportional to body weight, to ensure the positivity of the equation without losing the accuracy in the description.

The simplified equation is first used in the model from Chapter II, describing the dynamics of food intake and body weight, with adaptation of energy expenditure and regulation by leptin, ghrelin and glucose. This new model is analyzed and displays at least a single positive stable equilibrium. Thus, the new model is compared to the original model on its ability to reproduce and predict experimental data, from Chapter II and from experiments

of caloric restriction on rats for 16 weeks. Results are similar for both models, despite a reduced number of parameter values to estimate in the new model.

Then, we study the new model describing leptin resistance by analyzing the system. As in Chapter III, the new system displays between one and two stable positive equilibria: a healthy state and a leptin resistant and obese state, with a hysteresis. The new model is then confronted to experimental data of leptin infusion from [Pal and Sahu, 2003]. It is able to reproduce the observed evolution of food intake and body weight, with results similar or slightly better than the ones of the initial model of leptin resistance.

As the new models display the same results as the original models (from Chapters II and III), it is then possible to build a full model, combining the regulation of body weight and food intake dynamics by leptin, ghrelin and glucose, adaptation of energy expenditure and the dynamics of leptin receptors. This new system is based on the equations from the first models of Chapter IV, with minor modifications to integrate the effect of leptin receptors on the regulation of food intake.

# Chapter II

# A predictive model of the dynamics of body weight and food intake in rats submitted to caloric restrictions

In this Chapter, we reproduce the article, entitled "A predictive model of the dynamics of body weight and food intake in rats submitted to caloric restrictions", published in PLoS One in 2014 [Jacquier et al., 2014].

In this article, we developed a mathematical model, based on ordinary and delay differential equations, of the regulation of body weight (divided into fat mass and fat-free mass), food intake and energy expenditure by hormones (leptin and ghrelin) and glucose, in rats. This model is used in the case of caloric restrictions, consisting in different amounts of food available each day. Experiments were conducted for this study and are used to calibrate the model and test its predictive power. The purpose of this model is to study the impact of different food patterns on the evolution of body weight in rats, experimentally and by simulations, and to evaluate the importance of adaptation in energy expenditure in the case of caloric restriction. We summarize hereafter the contents of this article, presented in details in Sections II.1 to II.4.

Experiments were conducted on Wistar rats for 8 weeks, rats were divided into different groups: one group sacrificed on the first day to obtain initial biometric data, one control group receiving *Ad libitum* feeding and three restricted groups receiving a total of 80% of the normal diet, with different patterns of food distribution: the first group (H0) receiving a constant amount of food each day, the second (H1) a different amount each

week and the last (H4) a very low amount for 4 weeks and a high amount for the rest of the experiment. The evolution of food consumption and body weight were monitored during the duration of the experiment and biometric data were collected after 8 weeks. By the end of the experiment, significant differences appeared between the groups. As expected, body weight is significantly lower for the restricted groups compared to *Ad libitum* group. Groups H0 and H4 show significant differences in body weight and in the total amount of food eaten, despite receiving the same amount. These results indicate that some adaptations occurred in energy expenditure, relatively to the amount of food consumed and the pattern of food intake.

The mathematical model takes into account fat mass, fat-free mass, hunger (defined as the amount of food consumed in the absence of restriction), plasma leptin, plasma ghrelin, plasma glucose, rate of energy expenditure and available food. Fat mass and fat-free mass are described by two coupled equations adapted from a previous model of body weight dynamics in mice [Guo and Hall, 2009, 2011]. The change in body weight depends on the difference between energy intake and energy expenditure. Energy intake depends on hunger and food available while energy expenditure is defined as a linear function of fat mass and fat free mass with a basal energy expenditure. The rate of energy expenditure is assumed to adapt to the previously consumed food, and is described by a delay differential equation, which allows to compare the amount of food recently eaten (last $\tau$ days) to a "reference food", consisting in the mean food intake during the last $\tau'$ days. Leptin, ghrelin and glucose are described by simple equations, with production activated or inhibited by other variables and constant elimination/degradation rates. Hunger is regulated by leptin, ghrelin and glucose, relatively to the biological properties of these regulators of food intake: hunger increases with ghrelin but is inhibited by leptin and glucose.

In order to test the predictive power of the model, we used experimental data on two groups to estimate parameter values and to predict the evolution of body weight and food intake for the remaining groups, with only the initial condition and food available as inputs. We showed that our model is able to predict quite accurately the evolution of body weight and food intake observed experimentally. The model gives better predictions for body weight than a simplified model with a constant rate of energy expenditure, highlighting the importance of adaptation of energy expenditure in the case of caloric restrictions.

We slightly modified the published version of our manuscript when reproducing it in this chapter: we identified a minor error in the definition of the energy expenditure $EE$, used in Equations (II.1) and (II.2), that has been corrected. Consequently, Figures II.5 to II.9

have been updated as well as Table II.4.

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PLOS ONE

# A Predictive Model of the Dynamics of Body Weight and Food Intake in Rats Submitted to Caloric Restrictions

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**Abstract**

Dynamics of body weight and food intake can be studied by temporally perturbing food availability. This perturbation can be obtained by modifying the amount of available food over time while keeping the overall food quantity constant. To describe food intake dynamics, we developed a mathematical model that describes body weight, fat mass, fat-free mass, energy expenditure and food intake dynamics in rats. In addition, the model considers regulation of food intake by leptin, ghrelin and glucose. We tested our model on rats experiencing temporally variable food availability. Our model is able to predict body weight and food intake variations by taking into account energy expenditure dynamics based on a memory of the previous food intake. This model allowed us to estimate this memory lag to approximately 8 days. It also explains how important variations in food availability during periods longer than these 8 days can induce body weight gains.

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## II.1 Introduction

Body weight regulation has become a major concern in our societies. A classical case of body weight dysregulation – obesity – is characterized by an excessive accumulation of white adipose tissue due to an energy imbalance between the energy derived from consumed food and the energy expended to maintain life [Abrams and Katz, 2011; Flier, 2004; Kahn et al., 2006]. Because obesity is recognized as an important health hazard [World Health Organization, 2000], the causes of this imbalance have been extensively investigated in the past several years [Barsh et al., 2000; Friedman, 2000] with findings pointing out to peripheral as well as central mechanisms controlling food intake [Morton et al., 2006; Schwartz et al., 2000; Woods et al., 2000]. While feeding behavior – especially in human – can be difficult to assess, food intake behavior can be modulated by numerous factors, including but not restricted to nutrient signals – meal size and composition – and also orexigenic and anorexigenic hormones [Crespo et al., 2014]. Among these hormones, ghrelin [Cummings and Shannon, 2003; Higgins et al., 2007; Wren et al., 2001],

cholecystokinin (CCK) [Duca and Covasa, 2012], peptide YY [Duca and Covasa, 2012], glucagon-like peptide-1 (GLP-1) [Duca and Covasa, 2012] and leptin [Stephens et al., 1995], have been identified as the main endocrine regulators of food intake. Anorexigenic gut peptides (CCK, GLP-1 and peptide YY) are produced in response to the presence of nutrients in the gastro-intestinal tract; their production is sensitive to changes in food composition such as an increase in fat content [Covasa, 2010; Duca et al., 2013]. An increased level of ghrelin triggers feeding behavior and ghrelin production is decreased during the course of a meal [Beck et al., 2002]. On the other hand, leptin, a hormone secreted by adipose cells in proportion to white adipose tissue accretion, is known to trigger satiety [Friedman and Halaas, 1998].

All these hormones control the energy input. Yet, adaptation of the basal energy expenditure is another mechanism regulating food intake. It aims at reducing the difference between energy intake and the energy needed by the organism [Garrow, 1987]. The latter can be modified by changes in activity and/or by adaptive thermogenesis (particularly in brown adipose tissue) [Lowell and Spiegelman, 2000; Schwartz et al., 2000; Tremblay et al., 2013]. In cases of overfeeding, thermogenesis is increased and ATP is wasted by completing futile cycles [Wijers et al., 2009], while when underfeeding, energy expenditure is reduced to vital mechanisms [Evans et al., 2005]. This adaptation can prevent weight loss despite a reduced energy intake [Tremblay et al., 2013]. However it is not instantaneous and can be sustained, leading to important weight gains in individuals previously submitted to a strict diet. This is observed in humans and explains why body weight does not decrease linearly in time despite a constant reduction in caloric intake [Hall et al., 2011].

In normal conditions, these mechanisms should control weight variations. However some perturbations can destabilize this control. Our objective is to investigate mathematically whether variations in food availability could be the origin of such a destabilization.

Numerous mathematical or computational models describing metabolism regulation and body characteristics evolution exist in the literature. These models focus on different modelling scales, from cell to organism and from seconds to years [de Graaf et al., 2009]. Some models have been applied to animal subjects. Tam et al. [Tam et al., 2009] focused on physiological effects of leptin on energy homeostasis and food intake in mice. Guo and Hall [Guo and Hall, 2009, 2011] predicted dynamics of body weight and composition with respect to energy use in mice. Van Leeuwen et al.[van Leeuwen et al., 2002] studied the effect of food restriction on survival and body growth in mice. Other models have been applied to humans and were focused on energy use [Chow and Hall, 2008; Hall, 2010b]

or relationships between fat mass and fat-free mass [Hall, 2007; Horgan, 2011]. These models were used to describe either normal conditions, overfeeding or starvation [Hall, 2006, 2010a].

From the modelling point of view, feeding behavior and hunger have been relatively ignored. Although some results on the feeding dynamics correlate with body mass index [Periwal and Chow, 2006], no other modelling work has ever studied the impact of food availability dynamics on the feeding behavior and body weight regulation.

In this paper, we propose a mathematical model of body weight dynamics (divided in fat mass and fat-free mass), taking into account hunger, defined throughout this manuscript as the amount of food needed by the organism, leptin, ghrelin and glucose variations. Food intake is assumed to be regulated by the available amount of food and by hunger. As we focus on the influence of available and consumed food, we have chosen to consider only leptin (as an indicator of fat storage), ghrelin (representative of the volume of food intake) and glucose (proportional to the energy content of the diet) amongst all the factors influencing food intake. Unlike other published models, this system includes a memory of past food intake to model the adaptation of energy expenditure to caloric restrictions.

To challenge the model and find relevant parameter values, we conducted a simple feeding experiment on rats. One group received *Ad libitum* food. The time course of the available food for the three other groups was modified with three different frequencies, while maintaining an isocaloric diet during the entire experiment. We show that low frequency perturbations are very likely to induce weight gains and that our model is able to predict this feature.

## II.2 Materials and Methods

### II.2.1 Experimental procedures

#### II.2.1.1 Animal care

Animal experiments were performed under the authorization n°69-266-0501 (INSA-Lyon, DDPP-DSV, Direction Départementale de la Protection des Populations - Services Vétérinaires du Rhône), according to the guidelines laid down by the French Ministère de

| | D0 | AL | H0 | H1 | H4 |
|---|---|---|---|---|---|
| Body weight (g) | $333.8 \pm 15.9$ | $493 \pm 46.7$ | $410.5 \pm 24.1$ | $404.8 \pm 40.5$ | $444.5 \pm 16.4$ |
| Body length (cm) | $22.9 \pm 0.53$ | $26.4 \pm 0.51$ | $25.4 \pm 0.66$ | $25.1 \pm 0.75$ | $26 \pm 0.67$ |
| rWAT (g) | $4.24 \pm 1.49$ | $9.77 \pm 2.30$ | $8.82 \pm 3.80$ | $7.63 \pm 1.56$ | $9.58 \pm 2.61$ |
| Total WAT (g) | $17 \pm 4.6$ | $33.3 \pm 9.4$ | $29.2 \pm 9.7$ | $28.2 \pm 4.7$ | $32.9 \pm 5.4$ |
| iBAT (mg) | $287 \pm 39$ | $425 \pm 199$ | $365 \pm 71$ | $367 \pm 64$ | $390 \pm 153$ |
| Kidneys (g) | $2.37 \pm 0.59$ | $3.11 \pm 0.22$ | $2.56 \pm 0.04$ | $2.69 \pm 0.35$ | $2.70 \pm 0.34$ |
| Heart (g) | $0.96 \pm 0.09$ | $1.40 \pm 0.13$ | $1.16 \pm 0.05$ | $1.24 \pm 0.12$ | $1.19 \pm 1.13$ |
| Soleus (g) | $235 \pm 48$ | $248 \pm 37$ | $172 \pm 33$ | $203 \pm 24$ | $183 \pm 45$ |
| EDL (g) | $110 \pm 37$ | $165 \pm 44$ | $193 \pm 18$ | $188 \pm 12$ | $192 \pm 51$ |

**Table II.1** – Biometric data. Each column gives biometric data for the 5 groups: for all groups except D0 (group sacrificed on the first day of the experiment), data have been obtained at the end of the experiments.
WAT = White Adipose Tissue, rWAT= retroperitoneal White Adipose Tissue, iBAT = interscapular Brown Adipose Tissue, EDL = Extensor digitorum longus.

l'Agriculture (n° 87-848) and the E.U. Council Directive for the Care and Use of Laboratory Animals of November 24th, 1986 (86/609/EEC). COS (n° 69266257) holds a special license to experiment on living vertebrates issued by the French Ministry of Agriculture and Veterinary Service Department.

Thirty twelve-week-old Wistar rats were purchased from Janvier SA (Le Genest-Saint-Isle, France) and housed in an air-conditioned room at $24 \pm 1$ °C with a LD (light/dark) 12:12 cycle (light on at 6:30 am) with free access to food (2016C, 12.6 kJ/g, 66% carbohydrates, 12% fat, 22% proteins, Harlan, Gannat, France) and water.

Rats were randomly separated into 5 groups (D0, AL, H0, H1 and H4) of 6 individuals (no significant difference of initial body weight was found between these groups : $p$-$value$=0.26). Each rat was identified and housed individually throughout the protocol.

The group D0 was sacrificed on the first day of the experiment (as described below), so the initial biometric data of the rats are available, including body weight, body length, white adipose tissue mass, brown adipose tissue mass, muscles and organs weights (see Table II.1). At the end of the experiment (i.e. 8 weeks) the other rats were sacrificed to obtain the same data. Blood samples were collected at the same time for further analyses. The total body lipid content can easily and accurately be predicted from the gravimetric determination of the retroperitoneal fat deposits [Newby et al., 1990]. Thus the retroperitoneal fat pads weights (rWAT) were used to estimate the total body lipid content ($L$ in grams), using the formula $L = 7.96\text{rWAT}+3.13$ [Newby et al., 1990].

| Group | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 |
|---|---|---|---|---|---|---|---|---|
| **AL** | *Ad libitum* | | | | | | | |
| **H0** | 20 g | 20 g | 20 g | 20 g | 20 g | 20 g | 20 g | 20 g |
| **H1** | 15 g | 25 g | 10 g | 10 g | 30 g | 35 g | 5 g | 30 g |
| **H4** | 10 g | 10 g | 10 g | 10 g | 30 g | 30 g | 30 g | 30 g |

**Figure II.1** – Daily available food per rat in each group. Changes of quantities occur each week, except for group AL which was not submitted to caloric restriction. At the end of the 8-weeks experiment, each rat in groups H0, H1 and H4 will have received 1120g of food. Consumed food is not always equal to this amount but is recorded every day.

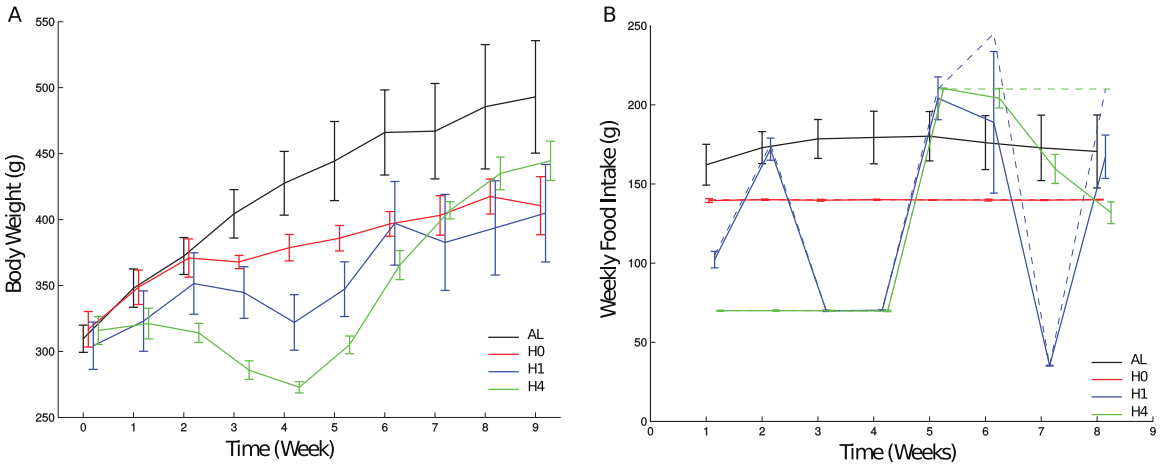


**Figure II.2** – Body weight and food intake evolution. Temporal evolution of body weights (in grams) for each group (mean± sd): AL (black), H0 (red), H1(blue) and H4 (green). A small offset has been added to the time points to ease the reading. **B)** Evolution of consumed food (straight lines, mean ± sd) weekly by each rat (in each group: AL (black), H0 (red), H1(blue) and H4 (green)) compared to the available food (dashed lines). Group H0 consumed all its available food for the duration of the experiment while groups H1 and H4 had leftovers.

Rats from groups AL, H0, H1 and H4 were individually housed and received chow diet for 8 weeks in different quantities each day (see Fig. II.1). The control group AL received *Ad libitum* food (approximately 25g per rat per day). *Ad libitum* food also corresponds to the diet before the beginning of the experiment in each group. The other groups (H0, H1, H4) were submitted to a restriction in caloric availability corresponding to 80% of *Ad libitum* diet. This reduction should theoretically avoid leftovers, as this amount is below normal consumption, in order to have a better control on food intake.

The pattern of food distribution was not the same for these three groups. Group H0 received the same amount of food every day for 8 weeks. For the H1 group the food was randomly allocated for each week of the experiment. Group H4 was submitted to an

important restriction for 4 weeks followed by an excess of food for the remaining 4 weeks. The amount of food given each day is reported in Fig. II.1. The remaining food was measured and removed each day to determine the food really consumed (see Fig. II.2 B). Great care was taken to ensure that most of the food was either eaten or removed and not wasted. In a preliminary experiment, we determined that food spillage only accounts for $6.9 \pm 1,0\%$ of the total food intake. Therefore it was considered to be negligible.

During the experiment, the beginning of the week (the day the food availability was changed) was set on Tuesday and rats were weighted every Friday morning. This protocol tends to minimize and to separate the effects of stress due to changes in food availability and stress due to weighting.

#### II.2.1.2 Sacrifice, blood and tissue collection

Animals were deeply anesthetized with sodium pentobarbital (60 mg/kg ip), blood ($\sim 5$ mL) was collected through puncture of vena cava on heparinized syringe and centrifuged 2 min at 8000 g. Plasma samples were snap frozen in liquid nitrogen and stored at -80 °C until analysis. Liver, heart, kidneys, gastrocnemius muscles, epididymal, retroperitoneal and subcutaneous inguinal white adipose tissue (WAT) were dissected out according to anatomical landmarks, weighed to the nearest milligram, snap frozen in liquid nitrogen and stored at -80°C. Total WAT mass was calculated as the sum of the mass of epididymal, retroperitoneal and subcutaneous inguinal WAT deposits.

Individual data is freely available upon request.

#### II.2.1.3 Biochemical analysis

Plasma ghrelin and leptin assays were performed using immunoassays (acylated rat/mouse ghrelin # A05117 and rat/mouse leptin EIA # A05176, Cayman, SpiBio, Montigny le Bretonneux, France) according to the manufacturer's recommendations. The detection limit and intra-assay coefficient of variation for ghrelin were 0.2 pg.mL$^{-1}$ and 11%, respectively. The detection limit and intra-assay coefficient of variation for leptin were 50 pg.mL$^{-1}$ and 4%, respectively. Blood glucose was measured using an automatic glucose monitor (Optium Xceed, Abbott, Rungis, France). All assays were performed at least in duplicate (see Table II.2).

| | D0 | AL | H0 | H1 | H4 |
|---|---|---|---|---|---|
| Ghrelin (pg.mL$^{-1}$) | nd | 43.30 ± 17.75 | 25.66 ± 14.65 | 78.48 ± 97.96 | 18.01 ± 9.88 |
| Leptin ( ng.mL$^{-1}$) | 4.34 ± 1.78 | 7.53 ± 2.72 | 3.60 ± 1.66 | 5.19 ± 1.66 | 7.07 ± 3.66 |
| Glucose ( mg.dL$^{-1}$) | 140.7 ± 11.6 | 148.7 ± 24.1 | 132.2 ± 8.9 | 121.4 ± 14.8 | 144.3 ± 21.2 |

**Table II.2** – Plasma hormones and glucose assays. Ghrelin, leptin and glucose concentrations in plasma in the control group and at the end of the experiment for groups AL, H0, H1 and H4 (mean ± sd, nd: not determined).

Individual data is freely available upon request.

### II.2.2 Mathematical model

In this section, the mathematical model is described (See Table II.3 for a description of all variables and Figure II.3 for a schematic representation of the system). This model focuses on fat mass and fat-free mass evolutions regulated by hunger and available food. Hunger is defined as the amount of food the system would consume were there no constraint on food availability. There exist multiple factors influencing food intake [Crespo et al., 2014; Duca and Covasa, 2012; Morton et al., 2006; Schwartz et al., 2000; Woods et al., 1998, 2000], yet we focus on 3 of them: leptin, ghrelin and glucose (which is highly correlated with insulin) as they regulate hunger at different time scales [Schwartz et al., 2000].

| Name | Symbol | Unit |
|---|---|---|
| food available | $a$ | kJ |
| hunger | $h$ | kJ |
| plasma ghrelin | $e$ | pg.mL$^{-1}$ |
| plasma glucose | $u$ | g |
| plasma leptin | $l$ | ng |
| fat mass | $S$ | g |
| fat-free mass | $W$ | g |
| rate of energy expenditure | $R$ | min$^{-1}$ |

**Table II.3** – Variables of the model with associated units and symbols.

Fat mass ($S$, in grams) and fat-free mass ($W$, in grams) are assumed to be produced depending on the instantaneous difference ($\Delta_E$) between energy intake and energy expenditure. To model this phenomenon we adapted the equations in [Guo and Hall, 2009, 2011] previously developed for a mouse model and we used the same notations: $\rho_S$ and $\rho_W$ denote the energy densities for fat mass and fat-free mass respectively (kJ.g$^{-1}$) and

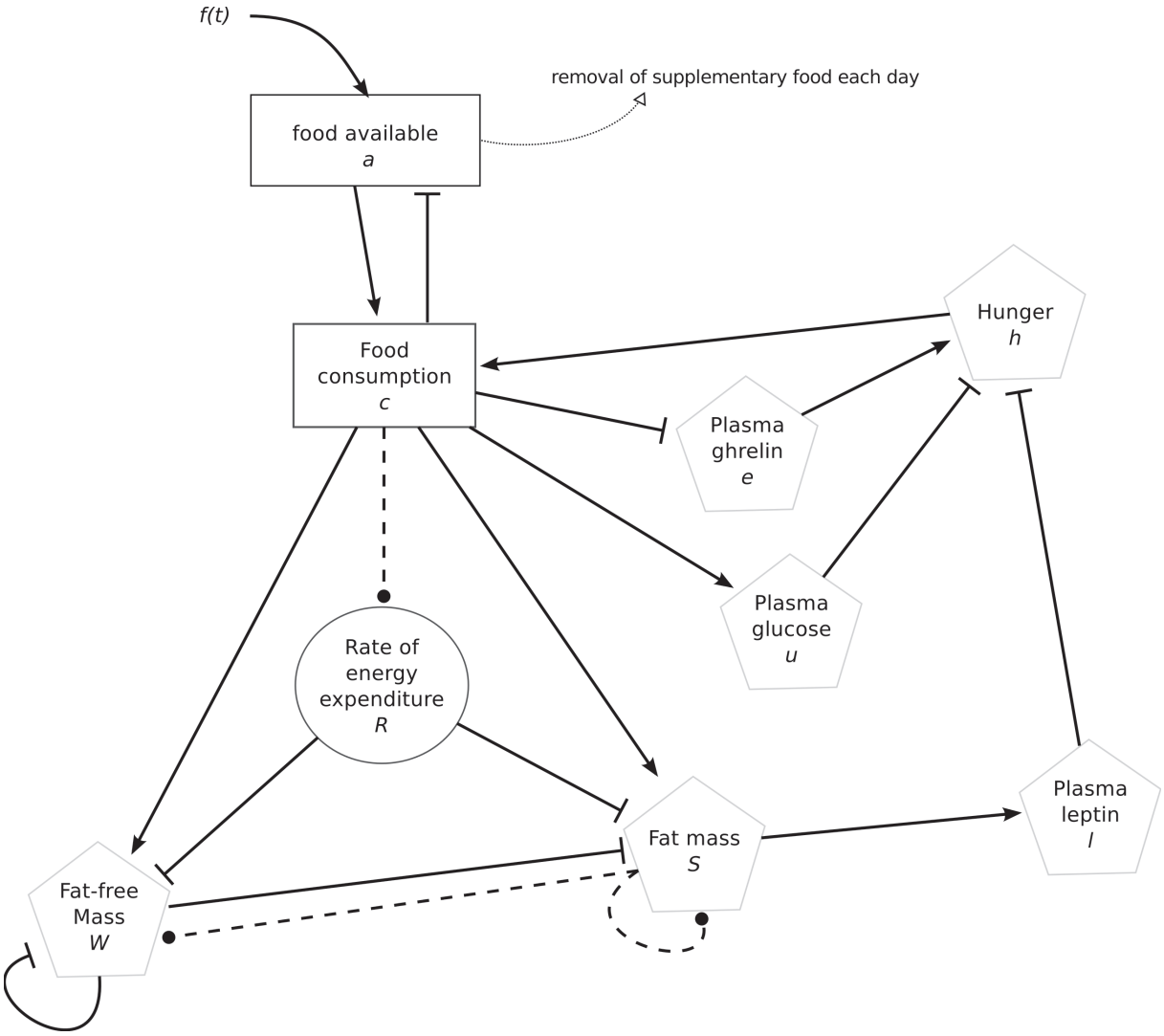


**Figure II.3** – Schematic representation of the model. Positive influences are represented by straight lines with arrows and negative influences by bar-headed lines. Relations whose effect can vary in time are represented by dashed lines with a dot at the end.

$\Delta_E$ the instantaneous difference of energy (kJ.min$^{-1}$). Evolutions of $S$ and $W$ are given by:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\Delta_E}{\rho_W x + \rho_S}, \tag{II.1}$$

$$\frac{\mathrm{d}W}{\mathrm{d}t} = \frac{\Delta_E x}{\rho_W x + \rho_S}, \tag{II.2}$$

where $x \equiv \mathrm{d}W/\mathrm{d}S = \zeta + \psi.\exp(\kappa.S)$ [Guo and Hall, 2009, 2011].

Energy intake ($EI$) is supposed to be a function of the caloric content of the diet. This food consumption per minute, denoted by $c(a, h)$ (kJ.min$^{-1}$), is assumed to be a function

of hunger $h$ (kJ) and available food $a$ (kJ). We assume $c(a, h)$ is equal to the minimum of $a$ and $h$ per unit of time. Hunger was defined as the amount of food needed by the system (see above). Hence, food consumption is either equal to hunger, when enough food is available or to the available food $a$.

Several formulae describe energy expenditure [Horgan, 2011; Nelson et al., 1992], using linear dependencies on body weight, fat mass and fat-free mass. In the current model, the energy expenditure ($EE$) is assumed to be a function of fat mass and fat-free mass, with a rate of energy expenditure $R$. The result is the amount of Joules lost per minute. We then define the energy balance $\Delta_E$ as:

$$\Delta_E = EI - EE = c(a, h) - R(\gamma_W W + \gamma_S S + \xi),$$

where $EI = c(a, h)$ and $EE = R(\gamma_W W + \gamma_S S + \xi)$.

One can note that fat-free mass has a negative feedback on itself and that fat-mass may have either a positive or negative feedback on itself, depending on the value of $\Delta_E$. Fat mass can have a positive feedback on fat-free mass, via $x$ (see equation (II.2)), since creation of fat mass leads to the creation of lean mass [Hall, 2007].

The evolution of the amount of available food $a$ (in kJ) depends on the input of food in the system $f$ (usually a given amount each day) and the consumption $c$. The available food $a$ satisfies

$$\frac{\mathrm{d}a}{\mathrm{d}t} = f(t) - c(a, h). \tag{II.3}$$

In order to describe variations in appetite, the model should take into account the evolution of factors influencing hunger. As previously mentioned, we focus on leptin, glucose and ghrelin concentration. The total plasma leptin $l$ (in ng) is assumed to be produced proportionally to the fat mass [Tam et al., 2009],

$$\frac{\mathrm{d}l}{\mathrm{d}t} = \gamma_2 S - \gamma_1 l. \tag{II.4}$$

Total glucose $u$ (g) and ghrelin concentration $e$ (pg.mL$^{-1}$) in plasma depend on the diet composition [Beck et al., 2002]. The glucose level increases with food intake as follows:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = \mu_1 c(a, h) - \mu_2 u. \tag{II.5}$$

Ghrelin production is inhibited in the presence of food in the stomach [Cummings, 2006],

$$\frac{\mathrm{d}e}{\mathrm{d}t} = \frac{\nu_2}{1 + \nu_1 c(a,h)} - \nu_3 e. \tag{II.6}$$

The hunger $h$ is regulated in the central nervous system, integrating signals from the rest of the body via circulating hormones [Morton et al., 2006; Schwartz et al., 2000]. Regulation of hunger is a complex system. The amount of circulating leptin as an indicator of body adiposity leads to a decrease in hunger [Schwartz et al., 2000], so we assume hunger decreases when leptin increases. The ghrelin concentration decreases when the stomach is full and the hunger follows the same variations [Cummings and Shannon, 2003] so we assume hunger increases when ghrelin increases. The effect of leptin and ghrelin is opposite, though they both have an action in the arcuate nucleus [Beck et al., 2002]. The hunger $h$ is also supposed to be a decreasing function of glucose level $u$ [Campfield and Smith, 1990]. The hunger $h$ was defined as the amount of Joules required by the system at any time, so the evolution of $h$ is given by:

$$\frac{\mathrm{d}h}{\mathrm{d}t} = \frac{\alpha_1 e}{1 + \alpha_2 l} - \beta(\alpha_3 + u)h. \tag{II.7}$$

System (II.1)-(II.7) takes regulations at short and long time scales into account. Variables directly linked to daily food intake such as ghrelin concentration and glucose level have an influence on a daily basis whereas leptin has an influence on a longer time scale.

### II.2.3 Adaptation of energy expenditure

The previously described model is well adapted when food is available *Ad libitum*. As the food consumed is always equal to hunger, the organism does not need to change relatively to environmental conditions and its rate of energy expenditure $R$ is therefore constant. In the case of caloric restrictions, energy expenditure is lowered to maintain the energy balance [McCarter and Palmer, 1992]. To take this phenomenon into account, we assume that the rate of energy expenditure $R$ depends on the food consumed, with a memory effect.

The rate of energy expenditure $R$ is known to adapt to the past food intake $c(a,h)$ [Evans et al., 2005]. As the food is supposed to be available on a daily basis, the mean food intake in the last $\tau$ days is compared to the mean food intake in the last $\tau'$ days (with

$\tau' > \tau$) to define the value of $R$. The "reference" food (food consumed between times $t - \tau'$ and $t$) is slowly modified accordingly, so $R$ is constant if the food intake doesn't change for at least $\tau'$ days. When food intake varies on short periods of time, the rate of energy expenditure $R$ is progressively modified to reduce the difference between these mean food intakes, with a rate of adaptation equal to $\epsilon$, as follows:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \epsilon \left( \frac{1}{\tau} \int_{t-\tau}^{t} c(a(v), h(v)) \mathrm{d}v - \frac{1}{\tau'} \int_{t-\tau'}^{t} c(a(v), h(v)) \mathrm{d}v \right). \tag{II.8}$$

This equation needs an initial condition $R_0$ which corresponds to the value of the rate of energy expenditure with a constant food intake equal to hunger (*Ad libitum* case).

Other factors influence energy expenditure [Garrow, 1987; Woods et al., 1998] such as plasma leptin, environment and aging [Greenberg and Boozer, 2000; McCarter and Palmer, 1992]. Nevertheless this model focuses only on the effect of caloric variations as it is the easiest parameter to measure and manipulate experimentally.

### II.2.4 Parameter estimation

System (II.1)–(II.8) uses 23 parameters whose values are essential to the relevance of the simulation results. Amongst these, the 4 parameters of hormone production and degradation are taken from the literature (see Table II.4 for a summary of units and origins of parameters of the model). Food-relative parameters depend on the composition of the chow diet.

To estimate the 12 remaining parameters, we used the final fat mass and the evolution of body weight of each individual rat from groups AL and H1. Parameter values were obtained by minimizing the residual sum of squares (RSS) of observed data compared to simulation results using the Nelder-Mead algorithm [Nelder and Mead, 1965]. We then used these parameter values to test the predictive capacity of our model against data from groups H0 and H4.

In the AL case, *Ad libitum* food implies that food intake $c$ is always equal to $h$ and the rate of energy expenditure $R$ is constant. Consequently, energy expenditure only depends on fat mass $S$ and fat-free mass $W$. We have access to the experimentally consumed food, so we use this value to explicitly determine the evolution of $h$. Hence equations (II.1) and (II.2) are decoupled from the other equations and we consider them as an independent subsystem.

| Parameter | Value | Unit | |
|---|---|---|---|
| $\nu_1$ | 1.52 | $\text{min.kJ}^{-1}$ | experiments |
| $\nu_2$ | 0.4025 | $\text{pg.mL}^{-1}\text{.min}^{-1}$ | experiments |
| $\nu_3$ | 0.007 | $\text{min}^{-1}$ | Vestergaard et al. [2007] |
| $\mu_1$ | 0.039 | $\text{g.kJ}^{-1}$ | diet composition |
| $\mu_2$ | 0.007 | $\text{min}^{-1}$ | Cobelli et al. [1984] |
| $\gamma_1$ | 0.074 | $\text{min}^{-1}$ | Zeng et al. [1997] |
| $\gamma_2$ | 0.126 | $\text{ng.g}^{-1}\text{.min}^{-1}$ | Zeng et al. [1997] and experiments |
| $\gamma_W$ | 0.628 | $\text{kJ.g}^{-1}$ | Guo and Hall [2011] |
| $\gamma_S$ | 0.125 | $\text{kJ.g}^{-1}$ | Guo and Hall [2011] |
| $\rho_W$ | 7.5 | $\text{kJ.g}^{-1}$ | Guo and Hall [2011] |
| $\rho_S$ | 39.3 | $\text{kJ.g}^{-1}$ | Guo and Hall [2011] |
| $\xi$ | 21.96 | kJ | fit step 1 |
| $\zeta$ | 2.651 | — | fit step 1 |
| $\psi$ | $1.88 \times 10^{-4}$ | — | fit step 1 |
| $\kappa$ | $1.114 \times 10^{-9}$ | $\text{g}^{-1}$ | fit step 1 |
| $R_0$ | $7.224 \times 10^{-4}$ | $\text{min}^{-1}$ | fit step 1 |
| $\alpha_1$ | $4.61 \times 10^{-9}$ | $\text{mL.kJ.min}^{-1}\text{.pg}^{-1}$ | fit step 2 |
| $\alpha_2$ | $5.98 \times 10^{-4}$ | $\text{ng}^{-1}$ | fit step 2 |
| $\alpha_3$ | $5.76 \times 10^{-4}$ | g | fit step 2 |
| $\beta$ | $5.839 \times 10^{-11}$ | $\text{min}^{-1}\text{.g}^{-1}$ | fit step 2 |
| $\epsilon$ | $1.92 \times 10^{-7}$ | $\text{kJ}^{-1}$ | fit step 3 |
| $\tau$ | 1 | day | fit step 3 |
| $\tau'$ | 16 | day | fit step 3 |

**Table II.4** – Values of the parameters used in the model and associated units. When the parameter is taken from the literature, the corresponding reference is indicated.

In a first step, applying the minimization algorithm to this subsystem leads to an estimation of the 4 parameter values relative to equations (II.1) and (II.2). Then, in a second step, we estimate the remaining parameter values using equations (II.1), (II.2), (II.4), (II.5), (II.6) and (II.7) and the previously determined parameters. To estimate the parameters relative to $h$ in equation (II.7) we once again use data from group AL. However, this time, $c$ was determined by the values of $a$ corresponding to *Ad libitum* food and $h$ is given by (II.7). As $R$ is supposed to be constant over group AL, the system used here was composed by all equations except (II.8). Finally, those parameters relative to the rate of energy expenditure $R$, were estimated in a third step, using experimental data from hypocaloric group H1. We then used the whole system of equations and parameter values previously estimated for AL.

Parameter estimation is detailed as follows:

Step 1 Only equations (II.1) and (II.2) are used. The input of the system is the experimentally determined consumed food $c$ for group AL. We assume $R$ is equal to $R_0$ when *Ad libitum* food is available. As we have a value for $c$, it is not necessary to describe the variations of hunger and hormones so the only dynamical variables of the subsystem are $S$ and $W$. The RSS between outputs of the model (predicted body weight and fat mass) and experimental data (body weight and fat mass) is minimized for each individual rat from group AL. This leads to an estimation of the parameters $\xi$, $\kappa$, $\psi$, $\zeta$ and the basal rate of energy expenditure $R = R_0$.

Step 2 Equations (II.1), (II.2), (II.4), (II.5), (II.6) and (II.7) are used with experimental data from group AL. Parameter values determined at step 1 are used at this step. The rate of energy expenditure $R$ is still supposed to be constant as the food is *Ad libitum*, with $R = R_0$ determined in the previous step. In this step, $c(a, h)$ is supposed to be equal to $h$ as $a$ is always higher than $h$ (to take unlimited food into account). This leads to an estimation of the parameter values relative to the hunger $h$: $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\beta$.

Step 3 For the last step, the whole system is used. Both the pattern of food availability and experimental data from group H1 are used, with five initial days of *Ad libitum* food to be consistent with the experiment. Parameters determined at steps 1 and 2 are used. As the H1 rats are supposed to adapt to the reduced and varying amount of food available, this allows to estimate the parameters associated with energy expenditure variations: $\epsilon$, $\tau$ and $\tau'$ in equation (II.8). Initial condition for $R$ is chosen to be equal to $R_0$ determined at step 1 as initial food is *Ad libitum*.

Akaike Information Criteria (AIC) was computed to compare the ability of the current model and of a model without memory (using equations (II.1)-(II.7) and $R$ constant as for the AL case) to reproduce the data. $AIC = n \ln(RSS/n) + 2k$ with $n$ the number of points used to evaluate the results, $RSS$ the residual sum of squares and $k$ the number of estimated parameters.

Approximate bayesian computation (ABC) was used to calculate a distribution of the computed parameter values, starting with uniform sampling around optimized parameters. Runs with a residual sum of squares smaller than a certain level $\mathrm{RSS}_{opti}$ (defined using the result of the optimization process) were selected; here the threshold was equal to 1.3 $\mathrm{RSS}_{opti}$(see Table II.5 for means and standard deviations of these distributions).

| | $\epsilon$ (kJ$^{-1}$) | $\tau$ (day) | $\tau'$ (day) |
|---|---|---|---|
| mean | $1.01\times10^{-8}$ | 1.3 | 8.4 |
| standard deviation | $0.74\times10^{-8}$ | 0.9 | 6.1 |

**Table II.5** – Approximate bayesian computation of parameters. Mean and standard deviation of selected runs of the ABC (RSS $<$ 1.3 RSS$_{opti}$) for parameters relative to the memory of the system ($\epsilon$, $\tau$ and $\tau'$). Mean values are close to parameter values estimated with the optimisation process but with an important standard deviation around these values.

### II.2.5 Predictions

Following the estimation procedure (see previous paragraphs), the model was tested with the patterns of food input corresponding to the two other groups of rats (H0 and H4) to evaluate its predictive capacity. Parameter values determined for groups AL and H1 were used. As all the rats were supposed to be similar (same origin and age), we used the same parameter values for each group.

If another group (H0 or H4) was chosen at step 3 of the estimation procedure instead of H1, the set of parameters associated with $R$ was different. However the RSS for each set of parameters were close from one another. Hence, the simulated data will be better for the chosen group than it will for the other groups. The data from each group could be fitted individually to have better results but this would suppress the predictive capacity of the model.

### II.2.6 Statistical analysis

All results are presented in the form: mean $\pm$ standard deviation.

Normality of the samples was tested using Shapiro-Wilks test. Statistical comparison was performed using Mann-Whitney test for two groups, and an analysis of variance (ANOVA) for more than two groups. All analyses were performed using the R software (www.R-project.org).

## II.3 Results

### II.3.1 Food availability modifies body weight dynamics

We present in this section the results of the experiments performed on rats – see the "Materials and Methods" section for details. In addition to a control group (called AL for *Ad libitum*, $n = 6$), three groups of 6 rats had their food availability modified during the 8 week long experiment. All rats experienced *Ad Libitum* feeding conditions prior to the experiment. Figure II.1 describes the available food time course for groups H0, H1 and H4 characterised by periods of variations of 0, 1 and 4 weeks respectively. In order to ensure a controlled total food intake, these groups were hypocaloric (around 80% of AL's average intake). Group H0 was daily fed with a constant amount of food with no variations. Group H1 was daily fed with random and uncorrelated amounts of food around the average. The feeding pattern of group H4 basically corresponds to a fasting experiment for 4 weeks (less than 60% of the AL's average intake) followed by a refeeding in the following month. In the three hypocaloric groups, the total amount of food provided to each rat during the whole experiment was the same (1120g in 8 weeks corresponding to 14.07 MJ – see Figure II.1).

At the end of the experiment, individuals were sacrificed and fat mass, muscles masses and some organ masses were collected and weighted. Table II.1 displays the values along with an initial control group sacrificed on the first day of the experiment (called D0 for "day 0"). As expected, body weight is smaller for the groups with reduced food (H0, H1 and H4) compared to group AL ($p = 0.00086$) and different between the 4 groups ($p = 0.0008$ for the ANOVA). There is no significant evidence that distributions of body weights in each group are not normal ($p - values$ between 0.21 and 0.97).

As shown on Figure II.4 A, a difference in final body weight exists within hypocaloric groups H0, H1 and H4, the corresponding $p - value$ is slightly above the 5% threshold ($p-value = 0.0636$). Pairwise comparison yields significant differences between H0 and H4 ($p - value = 0.02056$) whereas total food consumption (see Figure II.4 B) is significantly different between the two groups ($p - value = 0.005$), in the *opposite direction*. Rats in group H4 have a higher body weight although they ate less food than rats from group H0. No such differences are observed for group H1.

These results suggest that an energy expenditure adaptation occurs according to the amount of food consumed. This is summarized on Figure II.4 C which shows the variations

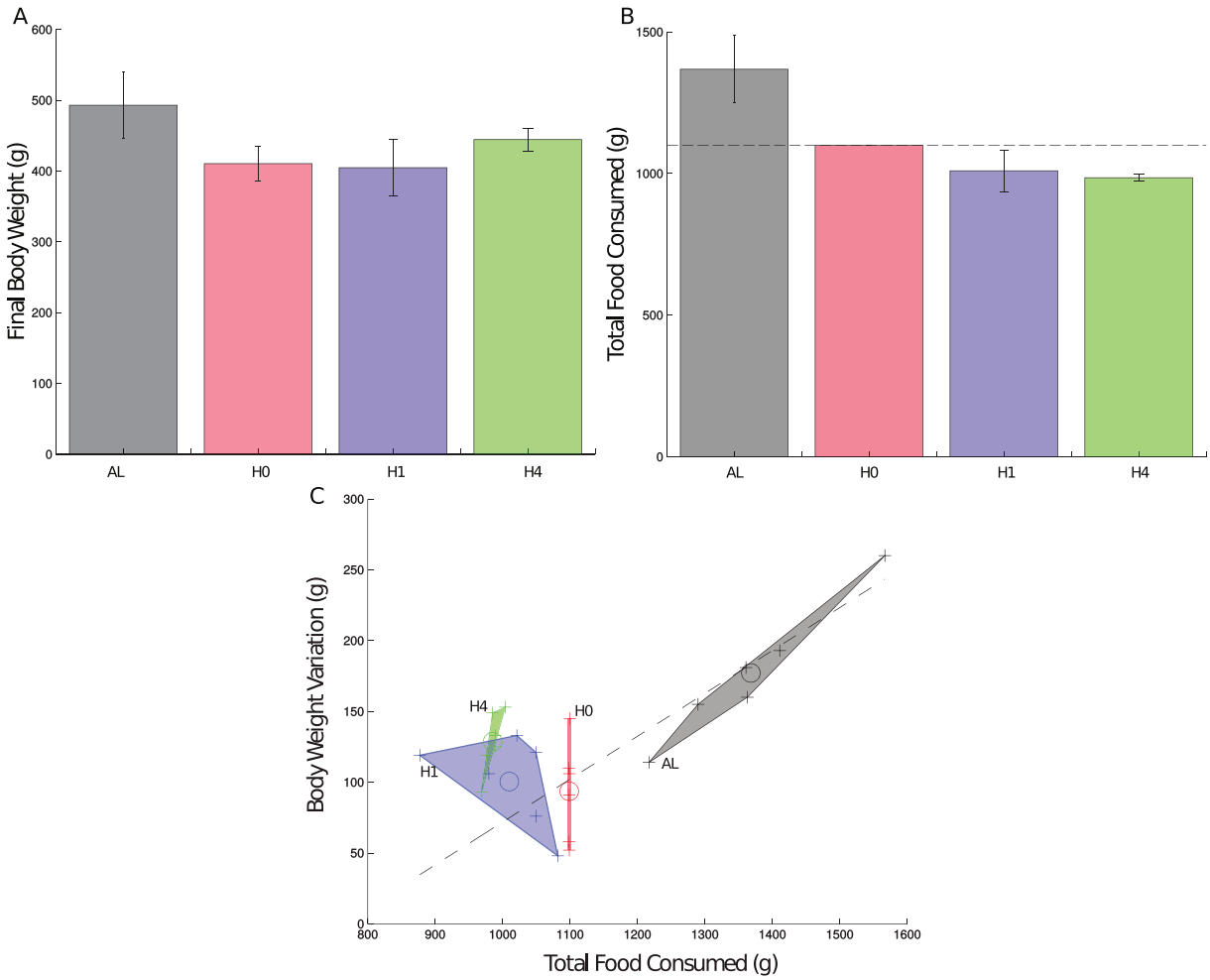


**Figure II.4** – Final experimental body weight and food intake. **A)** Final body weights at the end of the experiment for all groups. All hypocaloric groups (H0, H1, H4) are significantly different from group AL. Within hypocaloric groups, H4 is significantly different from H0. **B)** Total amount of food consumed in grams at the end of experiment (8 Weeks) for all groups (mean $\pm$ standard deviation). All hypocaloric groups (H0, H1, H4) consumed significantly less food than group AL. Within hypocaloric groups, H4 is significantly different from H0 and H1. Dashed line is the amount of the total food that was available for the hypocaloric group. **C)** Variation of body weight from the start to the end of the experiment versus the total amount of food consumed. Crosses indicate individual points, open circles are the averages and the whole group is described by its convex hull. The dashed line is the linear regression for the group AL ($p = 0.005$ and $R^2 = 0.88$). The slope is 0.3, indicating that the weight gain is equal to 30% of the weight of the consumed food.

of body weight during the experiment as a function of the total amount of food consumed. All data points are plotted and the convex hull has been coloured according to each group. Data for group AL closely follows a linear pattern with slope 0.3 which indicates that each gram of food consumed turns into 0.3 grams of body weight. The other groups do not follow the same pattern. Strikingly group H4 is well above the line indicating that its individuals ate less food but that a bigger fraction of it turned into body weight. The H1

pattern is somewhat similar but less significantly.

Body weight evolution is displayed on Figure II.2 A and is consistent with the food intake in Figure II.2 B albeit with a delay, as the increase or decrease is associated with the food intake in the previous week. As observed in previous studies, when presented with various amounts of food, rats adapt their eating pattern depending on past eating behavior. Our main experimental result is that rats adapt their energy expenditure by taking efficiently advantage of the available food when in fasting conditions and using more energy when overfed. This behavior results in different body weights for the same caloric intakes.

### II.3.2 Mathematical model of food intake and body weight evolution

We show in this section the predictive power of our model of feeding behavior and food intake dynamics. The model and the equations are presented in details in the "Materials and Methods" section.

Our model describes the evolution of hunger, leptin, ghrelin, plasma glucose which is correlated with insulin, energy expenditure and body weight, composed of fat and lean mass (see Table II.3 for a list of the variables and their units). This model allows to describe hunger, defined as the amount of food needed by the organism, by computing the dynamics of food intake in the short term. Energy expenditure is described as a function of the rate of energy expenditure, fat-mass and fat-free mass. It includes a delay equation describing the variations of the rate of energy expenditure $R$. The evolution of $R$ depends on the comparison of short-term food intake with long-term food intake (see equation (II.8)). Figure II.3 describes the components of the model and Table II.4 describes the parameters as well as their units. The fitting procedure used to determine some parameter values is fully described in the "Materials and Methods" section. It uses only AL and H1 as training data sets.

Figures II.5.AL and II.5.H1 show the results of the parameter estimation on groups AL and H1, illustrated on body weight evolution. Simulations are accurate for both groups, as expected from the parameter estimation process. In addition, the model correctly predicts the results on the validating data sets: good matches are obtained for both H0 (Figure II.5.H0) and H4 (Figure II.5.H4). Variations of predicted body weight for group H4 correlate with modifications of food availability and are close to experimental values.

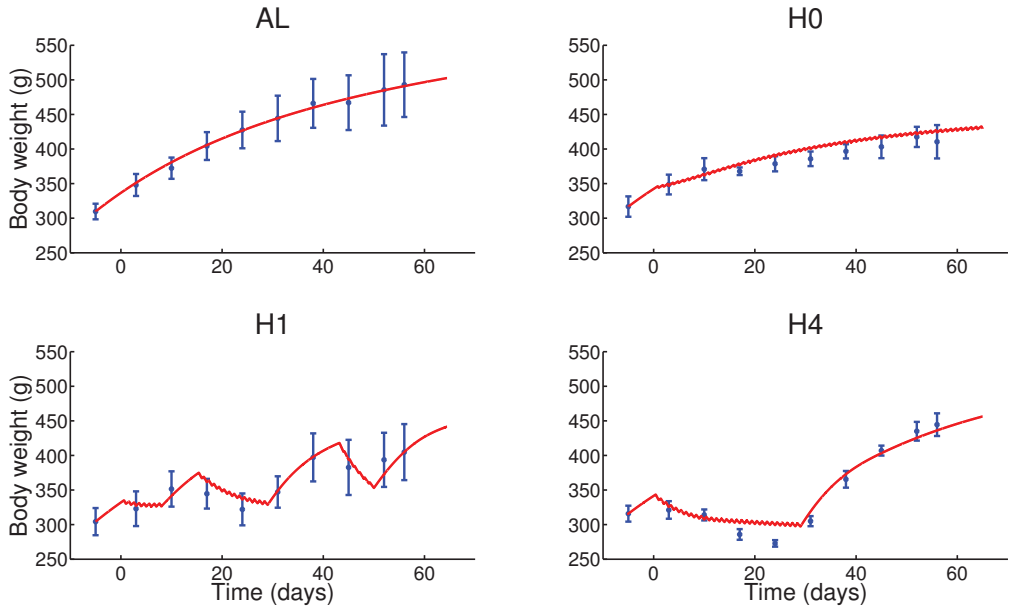


**Figure II.5** – Simulated evolution of body weight (red line) compared to experimental data (mean ± standard deviation in blue). In each group, the food input matches the experimental patterns and the first 5 days of the simulation were conducted with *Ad libitum* diet to be closer to the experiment. Parameter values were estimated with data from groups AL and H1 and predictions were made with these parameter values on groups H0 and H4. Top left: AL; top right: H0; bottom left: H1; bottom right: H4.

Small daily oscillations are observed in groups H0, H1 and H4, especially when available food is below hunger. These oscillations correspond to a daily pattern of food intake: while food is available, it is consumed, resulting in an increase in body weight, then the consumption is equal to 0 and the body weight decreases. In the case of group H4, the predicted body weight at the end of the period of restriction (week 4) is slightly higher than the actual data. As the amplitude of the restriction is important, the adaptation could be less efficient in reality than it is in the model. There are also other phenomena such as environmental conditions, excluded here for simplicity, that could influence this adaptation.

Food availability is the only input of the model (see "Materials and Methods" section). It is defined according to the experimental pattern, including the five days of *Ad libitum* diet at the beginning. Our model correctly predicts food intake pattern, as shown on Figure II.6. In particular, for groups H1 and H4, the model predicts leftover food as observed in reality. In all cases, predicted food intake is a close match to the experimental data.

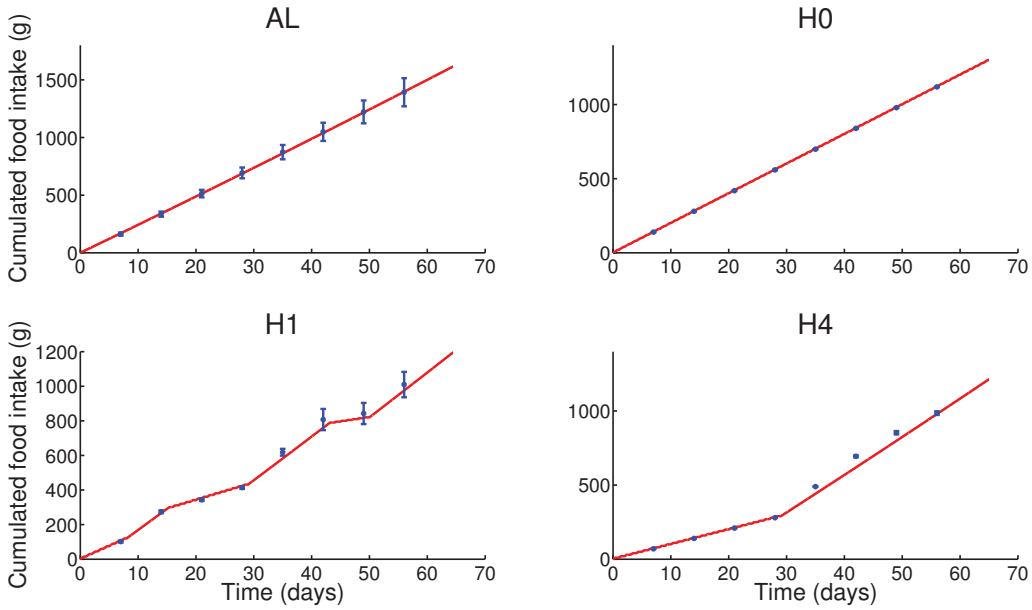


**Figure II.6** – Evolution of cumulated food intake predicted by the model (red curve) compared to experimental data (blue crosses: mean ± sd). Available food in the simulation corresponds to experimental patterns in each group, with *Ad libitum* diet in each group at the beginning. Results from groups AL and H1 correspond to the parameter estimation process while results for groups H0 and H4 correspond to predictions. Top left: AL; top right: H0; bottom left: H1; bottom right: H4.

### II.3.3 A metabolic memory is necessary to explain the observed data

The hypothesis that adaptation of the rate of energy expenditure is performed with a memory is included in equation (II.8) – namely the variable $R$ is modified with a memory of the food intake in the past. As explained in the previous section, this model leads to an accurate reproduction of the experimental data. To test the relevance of this memory in the system, simulations were run with a constant value of $R$ equal to the initial value $R_0$ of the rate of energy expenditure. The value of $R_0$ was obtained using the estimation procedure described in the section "Materials and Methods" without any memory of the past food intake and data from group H1. The model without memory was then applied to groups H0 and H4.

The values of the residual sum of squares are higher without memory than in the simulations with a non-constant $R$ for groups H0, H1 and H4. Moreover the data are no longer well explained and predicted without memory (see Figure II.7). Akaike Information Criteria allows us to objectively compare these two different models (see Table II.6). For

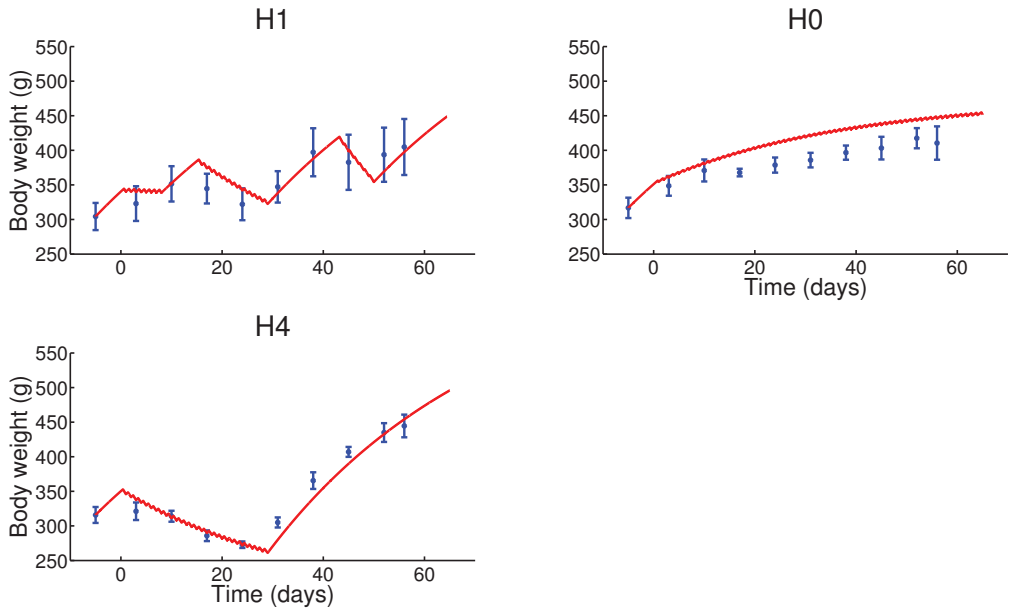


**Figure II.7** – Predicted body weight with a constant rate of energy expenditure $R$ compared to experimental results. Simulation for group H1 corresponds to the parameter estimation without memory (estimation of $R_0$ and other parameter values obtained for group AL). Predicted body weight in this case does not match experimental results. In particular, body weight is slightly overestimated for group H0 while in cases H1 and H4, the amplitude of variations is too important due to the absence of adaptation to food intake.

| | H0 | H4 | H1 |
|---|---|---|---|
| AIC with memory | 405.3 | 411.5 | 466 |
| AIC without memory | 467.3 | 397.2 | 472.1 |

**Table II.6** – Akaike Information Criteria (AIC) for groups H0, H4 and H1 to compare results of the model with and without memory. $AIC = n\ln(RSS/n) + 2k$ with $n$ the number of points used to evaluate the results, $RSS$ the residual sum of square and $k$ the number of parameters estimated. AIC is smaller in the model with memory, even if there are more parameters: this model is more adapted to explain these data.

each group, AIC is lower with memory than without, indicating that this model better explains our data despite the extra parameters to estimate. One may notice that better results could be obtained for the model without memory, by evaluating $R_0$ in each group separately, but the model would not be predictive anymore. For group AL, the memory did not impact the score, as expected: the rats are not submitted to caloric restrictions so they don't need to adapt their rate of energy expenditure to avoid weight variations.

### II.3.4 Hypothesis to explain body weight differences

The main result here is derived from the evolution of the rate of energy expenditure. The variations of $R$ are subjected to a delay equation that takes memory of past food intake into account. The model predicts the memory to be around 16 days (see Table II.4).

The important weight gain in group H4 during the last 4 weeks is then explained by the lag in the refeeding period when energy expenditure is still low (see Figure II.8) while food intake is at its highest (see Figure II.2 B). Due to the delay in the adaptation of the rate of energy expenditure, the difference between energy intake and energy expenditure is maximal during this period. In the H1 case, also submitted to important caloric variations, the period of 1 week is too short to modify the rate of energy expenditure in the same way as for group H4. The adaptation is then mitigated and the observed weight gain is less important than it is for group H4.

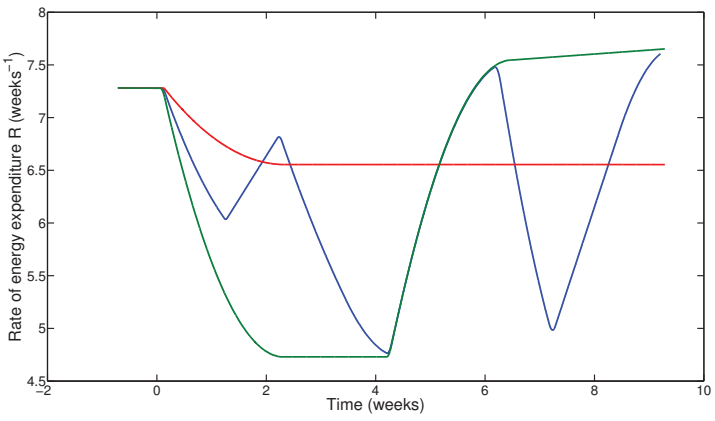


**Figure II.8** – Predicted rate of energy expenditure variations in the cases H0 (red), H1(blue) and H4 (green), starting from the same initial condition. $R$ value is stabilizing to a different value after a few days if the food pattern is followed for a long enough time ($>$ 16 days). Changes occur when the food availability is modified.

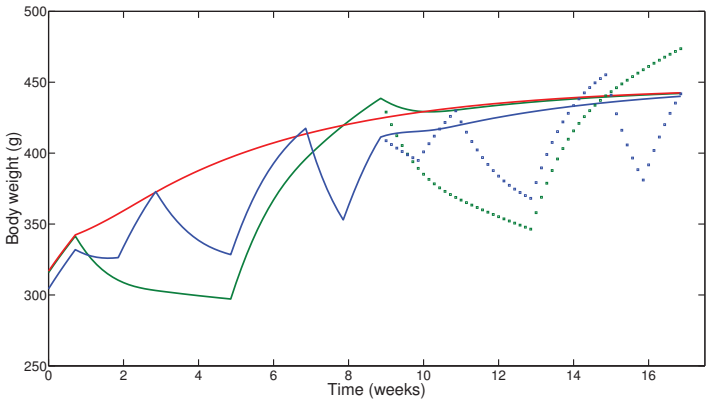


**Figure II.9** – Predicted body weight for a 16 weeks experiment with different combinations of food availability patterns. In blue H1 followed by H0 (line) or H1 (squares), in green H4 followed by H0 (line) or H4 (squares) and in red twice H0. In cases with H0 in the last 8 weeks (lines), the body weight tends to the same value, whatever the past variations.

The model was applied for 16 weeks (see Figure II.9), with H0 food pattern following H0, H1 or H4 experiment. With the same amount of food during the last 8 weeks for the three groups, the final predicted body weight tends to the same value regardless of the food pattern in the first 8 weeks. The lower food consumption for groups starting with H1 or H4 patterns does not impact this evolution. The adaptation to a constant amount of food intake leads to a fixed body weight after some time. Applying twice the same pattern (meaning the H1 diet for 16 weeks or the H4 diet for 16 weeks) leads to increases in body weights and fat mass, which reach elevated values(see Figure II.9). These variations with large amplitudes could have deleterious effects on the biological system, such as development of leptin or insulin resistances.

## II.4 Discussion

In this work, we showed that food availability fluctuations can trigger body weight variations that cannot be explained by differences in the overall energy intake. In our experiment, rats submitted to the same quantity of food but distributed differently over time exhibited significant weight differences. These differences were strongest when the period of variation was high – one month of low food availability followed by one month of important food availability.

In order to explain these results, we presented a new model of body weight dynamics,

describing hunger (defined as the amount of food needed by the organism), hormones and food availability dynamics. This model includes a delay equation describing variations of the rate of energy expenditure, which is adapting according to the memory of food intake. This delay equation was shown to be crucial.

After estimating the parameter values that best fit our experimental data, we showed that our model was able to both explain and predict food intake and body weight dynamics from our experimental results. We also showed that without the memory of food intake, the model cannot correctly reproduce the experimental data, which stresses that this adaptation is essential, in particular when food availability is low. Indeed, our model predicts that a period of caloric restriction leads to an increase in hunger and a decrease in the rate of energy expenditure. Ending these restrictions triggers a higher food consumption and a larger energy storage, with an increased rate of energy expenditure matching the food intake pattern. However this increase takes time to occur and during this delay period, a high amount of food is consumed while the energy expenditure remains low. We estimated a lag of 16 days which explains why quicker variations did not lead to any increase in weight. This provides a simple explanation for weight variations. A similar phenomenon is observed in humans and could explain why people submitted to very strict diets tend to gain more fat when they stop dieting, as their bodies have adapted to the reduced food consumption [Hall et al., 2011].

Although individual variability may play an important role when describing body weight variations and food intake dynamics, we did not focus on this aspect and rather considered an average behavior. The model proved its efficiency to describe the data. From the experimental results (Figure II.2), one may note that individual variability is globally initially low and increases with the duration of the experiment. Consequently, validation of the model's predictions on the evolution of body weight during a period of time greater than 8 weeks should be supported by additional experiments and could benefit from considering variability.

The model has largely ignored some phenomena such as aging processes which affect the rate of energy expenditure, appetite or sensitivity of the system to stimuli. Indeed feeding behavior can be extremely complex especially regarding food content and palatability. Also, leptin and insulin resistances are not included in this model but are known to have an influence on the regulation of appetite and storage of fat mass following an important weight gain. Including some of these phenomena could result in a better description of the system and help enhancing our understanding of the mechanisms behind these adaptations. Nevertheless, the approach developed in this work, based on innova-

tive mathematics and the use of a simple model, proved to be relevant to describe this physiological system.

# Chapter III

# A mathematical model of leptin resistance

In this Chapter, we reproduce the article entitled "A mathematical model of leptin resistance", published in Mathematical Biosciences in 2015 [Jacquier et al., 2015]. This model presents a possible mechanism of leptin resistance and allows to study the regulation of body weight by leptin and leptin receptors and the effect of different perturbations on the development of leptin resistance and obesity. It is based on the assumption that leptin is a regulator of its own receptors and that leptin receptors mediate the action of leptin on food intake. We hereafter summarize the contents of the article, presented in details from Section III.1 to Section III.4.

This mathematical model is based on the model developed in Chapter II, considering only leptin as a regulator of food intake, and without any adaptation of energy expenditure. A system of ordinary differential equations describes the dynamics of fat mass, leptin, leptin receptors and food intake. Fat-free mass dynamics are described by an algebraic equation depending on fat mass and initial conditions. Fat mass is modified relatively to the difference between energy intake and energy expenditure, defined as a function of fat mass. Leptin is produced proportionally to fat mass. Leptin is assumed to impact both the production and the degradation of leptin receptors, depending on its concentration: leptin receptors are downregulated by high leptin concentrations. The activation of leptin receptors by leptin, modelled by a Hill function, inhibits food intake. Some restrictions on parameter values are necessary to ensure the positivity of the solutions, in particular for fat mass.

The system displays one or three positive equilibria depending on parameter values, and is then monostable or bistable with an hysteresis. One equilibrium is characterized by low fat mass, low leptin and a high number of receptors, and corresponds to a healthy state. The other stable equilibrium is characterized by high fat mass, high leptin and low number of receptors and is designated as the obese and leptin resistant state.

The model is simulated with different perturbations, such as progressive variations in the stimulation rate of food intake and constant injection of leptin, to test their potential impacts on the development of leptin resistance. The development of leptin resistance corresponds to the evolution from a healthy state to an obese and leptin resistant one. Depending on the initial condition, an increase in the food intake stimulation rate can induce a change of equilibrium from the healthy state to the obese and leptin resistant state. Following this increase, a return to the initial value of the stimulation rate may not result in a return to the healthy state due to the hysteresis, the decrease must then be more important than the increase to return to the initial state. In the case of oscillations in the stimulation rate, which could correspond to a yo-yo diet, the solution can oscillate around the healthy state, around the obese state or between the two states, depending on the frequency and amplitude of the variation.

Constant leptin injection is a useful method to study the impact of leptin on leptin resistance. It is here modelled by adding a constant term to the equation for leptin and the results of the simulations are compared with experimental data on rats [Pal and Sahu, 2003]. Both in the experiment and in the simulations, which reproduce the experimental behaviour, leptin injection induces a decrease in food intake and body weight, that lasts for a few days. Then food intake increases again, and progressively returns to its initial value, at the end of the 16 days experiment. Body weight stabilizes for a few days before increasing again. In the simulation, leptin concentration has a 10-fold increase, starting at the beginning of the injection and remains constant afterwards. The number of receptors slightly increases before being importantly reduced, indicating the development of leptin resistance. Hence, by only considering a regulation of leptin receptors by leptin, the model reproduces experimental data on leptin injections and leptin resistance development.



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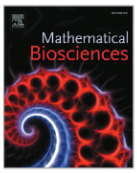

# A mathematical model of leptin resistance



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ABSTRACT

Obesity is often associated with leptin resistance, which leads to a physiological system with high leptin concentration but unable to respond to leptin signals and to regulate food intake. We propose a mathematical model of the leptin–leptin receptors system, based on the assumption that leptin is a regulator of its own receptor activity, and investigate its qualitative behavior. Based on current knowledge and previous models developed for body weight dynamics in rodents, the model includes the dynamics of leptin, leptin receptors and the regulation of food intake and body weight. It displays two stable equilibria, one representing a healthy state and the other one an obese and leptin resistant state. We show that a constant leptin injection can lead to leptin resistance and that a temporal variation in some parameter values influencing food intake can induce a change of equilibrium and a pathway to leptin resistance and obesity.

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# III.1 Introduction

Obesity is characterized by an excessive accumulation of adipose tissue resulting from an energy imbalance, where energy intake exceeds energy expenditure. Obesity has an important impact on health, with an increased mortality and is often associated with an increased risk of diseases including diabetes and hypertension [Friedman, 2000]. Causes of this dysregulation, including genetic and environmental conditions, and possible treatments are widely investigated [Guyenet and Schwartz, 2012; Naukkarinen et al., 2012].

Obesity is associated in most human and rodent cases to high concentrations of leptin in plasma [Friedman, 2000; Myers et al., 2010; Schwartz et al., 1996a; Zhang and Scarpace, 2006]. Leptin is a hormone produced by adipocytes (fat cells), regulating food intake and energy expenditure, known for its role as an indicator of the amount of fat storage in the organism [Friedman, 1998, 2014]: leptin concentration is proportional to fat mass. Increased leptin induces an inhibition of food intake. Leptin is thus involved in mechanisms regulating body weight [Friedman and Halaas, 1998].

Food intake is a complex process, regulated by a wide variety of oral and post-oral signals [Morton et al., 2006, 2014; Schwartz et al., 2000], including hormones ghrelin [Wren et al., 2001], cholecystokinin [Duca and Covasa, 2012] and leptin [Friedman and Halaas, 1998] to name a few (see for instance [Crespo et al., 2014] for details on food intake regulation by hormones). Leptin is known as the main regulator of food intake and energy expenditure. Circulating in plasma, leptin crosses the blood-brain barrier and reaches the arcuate nucleus of the hypothalamus where it binds to specific receptors. Activation of leptin receptors by leptin induces signaling cascades which negatively regulate food intake. Leptin also seems to influence its own impact on food intake regulation by regulating the expression of its cognate receptors [Martin et al., 2000; Myers et al., 2008; Pal and Sahu, 2003; Wilsey and Scarpace, 2004; Zhang et al., 1997] (see Figure III.1 for a schematic representation).

There exist different types of leptin receptors [Tartaglia, 1997], in particular LepRa and LepRb [Bjørbaek et al., 1997]. LepRa receptors may play a role in the transport of leptin from plasma to cerebrospinal fluid [Banks et al., 1999; Golden et al., 1997; Lynn et al., 1996]. LepRb receptors are located in the hypothalamus and are responsible for activation of the food intake and energy expenditure regulation pathways [Allison and Myers, 2014; Banks et al., 2000a].

Leptin resistance corresponds to the system's inability to integrate leptin signals in food intake regulation. The resistance can occur either at the blood-brain barrier (leptin transport to the brain is reduced [Caro et al., 1996]) or in the hypothalamus (reduced amount of receptors in the hypothalamus [El-Haschimi et al., 2000]). Leptin resistance has been observed in obese individuals: whereas intravenous injections of leptin lead to a decrease in food intake in healthy subjects, less important decrease or no decrease at all are observed in obese individuals [Widdowson et al., 1997]. When the resistance is located in the hypothalamus, an injection of leptin directly into the cerebrospinal fluid is not able to reduce food intake [Halaas et al., 1997]. Leptin resistance is then characterized by high concentrations of leptin in the brain that lead to a decrease in leptin receptors in the hypothalamus, the system has then a lower sensitivity to leptin and does not regulate food intake as well as it should. It results in increased food consumption and increased body fat, which produces more and more leptin, leading to a vicious cycle and in some cases, to the development of obesity [Scarpace et al., 2005; Zhang and Scarpace, 2006].

The purpose of this work is to propose a theoretical model of leptin resistance development, and to qualitatively study the dynamics behind the development of leptin resistance and its influence on food intake and body weight. To our knowledge, there is no math-

ematical model describing the emergence of leptin resistance. However, there exist a wide variety of models describing the regulations of body weight and metabolism (see [de Graaf et al., 2009] and the references therein, [Chow and Hall, 2014; Horgan, 2011]). These models, based mainly on ordinary differential equations, consider different mechanisms of regulation such as energy use in humans [Chow and Hall, 2008; Hall, 2010a; Horgan, 2011] or in rodents [Guo and Hall, 2009, 2011]. In humans, Horgan [Horgan, 2011] described the regulation of body weight regulated only by itself and food intake with a discrete stochastic model and concluded that body weight remains around a fixed value if the mean food intake and physical activity are constant. In [Chow and Hall, 2014], Chow and Hall studied the impact of stochastic fluctuations in food intake on body weight evolution and concluded that short-term fluctuations in food intake have a limited impact on body-weight. Guo and Hall [Guo and Hall, 2009, 2011] developed an ordinary differential equation model based on laws of energy conservation to predict changes in body weight and energy expenditure, using only energy intake, and applied this model to mice.

Other models considered the effects of hormones involved in food intake regulation in rodents [Jacquier et al., 2014; Tam et al., 2009]. Tam et al. [Tam et al., 2009] proposed a model, based on a system of ordinary differential equations, of metabolic regulation by leptin and compared normal regulation to leptin resistance. The latter has been modeled as a modification in parameter values involved in the transport of leptin into the brain and in the regulation of food intake, yet the authors did not consider the emergence of leptin resistance. There also exist models describing insulin resistant systems, linked to the development of diabetes [Gaetano et al., 2008; Topp et al., 2000]. Topp et al. [Topp et al., 2000] modeled the dynamics of blood glucose, insulin and $\beta$-cell mass, using a system of ordinary differential equations, and studied perturbations, such as insulin resistance, that can lead to diabetes.

Our objective is to present a simple theoretical model of leptin resistance, taking into account leptin concentration, leptin receptors density, food intake and body weight. Based on previous models of body weight dynamics in rodents [Guo and Hall, 2009, 2011], the main assumption of our model will be that leptin both up and down regulates leptin receptor expression – by positively regulating receptor degradation, and by also positively regulating receptor production. Activation of leptin receptors by leptin leads to the regulation of food intake, which influences body weight evolution. Leptin resistance is characterized by a state of the system with a high level of leptin which does not induce a loss of fat mass. The development of leptin resistance corresponds to the dynamical

evolution of the system from a healthy state to a leptin resistant state. We study the existence of equilibria of the system and analyze their stability. We describe the model qualitative behavior in steady conditions and for different cases of biologically relevant perturbations, such as an injection of leptin or modifications of parameter values, which can lead to the development of leptin resistance and obesity.

## III.2 Methods

### III.2.1 Mathematical model

In this section, the mathematical model is described. It focuses on fat mass, fat-free mass and food intake evolution and takes into account plasma leptin and leptin receptor dynamics to mediate food intake. Table III.1 provides a list of variables and their units, Table III.2 a a list of parameters, and Figure III.1 a schematic representation of the system.

We present below the equations of our model and the assumptions that led to this model. The main assumptions are listed hereafter:

(A1) Fat mass and fat-free mass dynamics follow the experiment-based relationship in mice described in Guo and Hall [Guo and Hall, 2009, 2011].

(A2) Energy expenditure is supposed to be a linear function of fat and fat-free mass [Jacquier et al., 2014; Nelson et al., 1992].

(A3) Leptin is produced proportionally to fat mass [Tam et al., 2009].

(A4) Leptin concentration regulates leptin receptors dynamics (it impacts both their production and their degradation) [Martin et al., 2000; Zhang et al., 1997].

(A5) Leptin-mediated activation of leptin receptors is described in terms of occupation theory (Hill function with a maximal response proportional to the density of receptors) [Kenakin, 2004; Ruffolo, 1982; Stephenson, 1956].

(A6) Activated leptin receptors are the main regulators of food intake [Myers et al., 2008].

Fat mass $FM$ (in grams) and fat-free mass $FFM$ (in grams) dynamics are adapted from the standard model of fat-free and fat mass dynamics proposed by Guo and Hall [Guo and Hall, 2009, 2011]. This model describes changes in body composition as a function

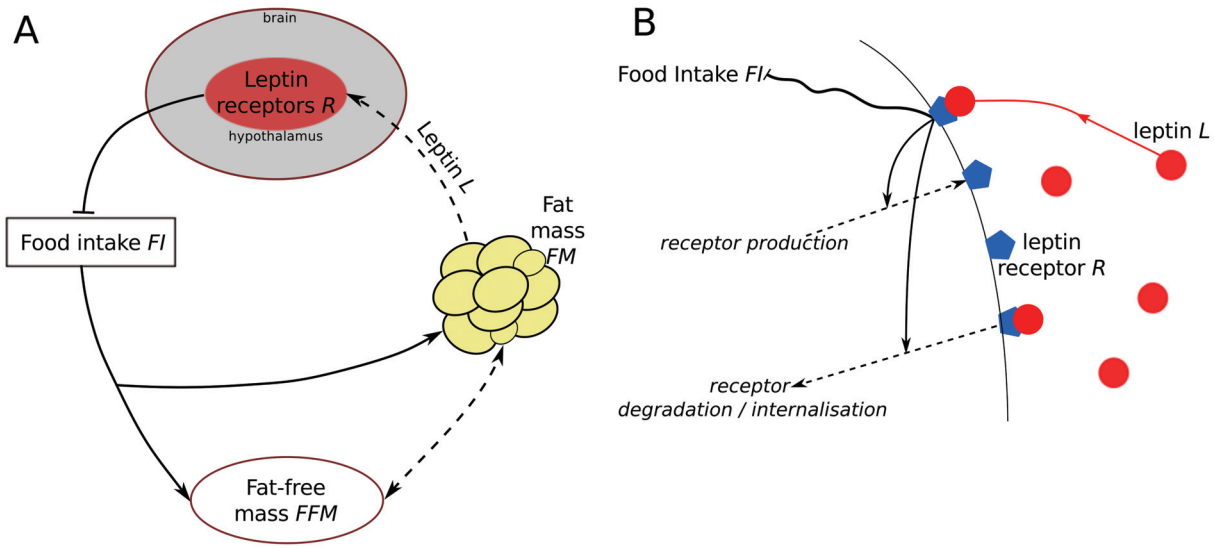


**Figure III.1** – A. Schematic representation of the leptin-mediated regulation of food intake at the scale of the whole system. Lines represent the action of the source on the target: straight lines with arrows display positive actions, bar-headed lines display inhibitions and dashed lines display actions that can be positive or negative depending on the parameter and variable values. B. Action of leptin (in red) on receptors (in blue) in the hypothalamus. Leptin binding on its receptors inhibits food intake (see A) and triggers a regulation of the production and degradation of receptors [Martin et al., 2000; Myers et al., 2008; Pal and Sahu, 2003; Wilsey and Scarpace, 2004; Zhang et al., 1997].

| Variable | | Unit |
|---|---|---|
| Fat mass | $FM$ | $g$ |
| Fat-free mass | $FFM$ | $g$ |
| Plasma leptin concentration | $L$ | $ng.mL^{-1}$ |
| Density of leptin receptors | $R$ | $mol.L^{-1}$ |
| Food intake | $FI$ | $g$ |

**Table III.1** – Variables of the model, their notations and units.

of energy dynamics in mice, based on fitting of experimental data (Assumption (A1)). Variations of $FM$ and $FFM$ are correlated with the difference between energy intake ($EI$) and energy expenditure ($EE$), $EI - EE$ ($kcal.min^{-1}$), which is a function of fat mass, fat-free mass and food intake (see Equation (III.3)) [Jacquier et al., 2014], as follows

$$\frac{\mathrm{d}FM}{\mathrm{d}t} = \frac{EI - EE}{\rho_{FFM}\Omega + \rho_{FM}}, \tag{III.1}$$

$$\frac{\mathrm{d}FFM}{\mathrm{d}t} = \frac{\Omega(EI - EE)}{\rho_{FFM}\Omega + \rho_{FM}}, \tag{III.2}$$

| Parameter | Unit | Default value |
|---|---|---|
| $\gamma_L$ | $ng.mL^{-1}.g^{-1}.min^{-1}$ | 0.5 |
| $\delta_L$ | $min^{-1}$ | 0.8 |
| $\gamma_R$ | $mol.L^{-1}.min^{-1}$ | 2.5 |
| $\delta_R$ | $min^{-1}$ | 0.9 |
| $\lambda_{R1}$ | $mL.ng^{-1}$ | 0.21 |
| $\lambda_{R2}$ | $mL^2.ng^{-2}$ | 0.05 |
| $\delta_{FI}$ | $min^{-1}$ | 1.2 |
| $\gamma_{FI}$ | $g.min^{-1}$ | 2.3 |
| $\phi$ | $L.mol^{-1}$ | 1 |
| $\theta$ | $ng.mL^{-1}$ | 3 |
| $n$ | $N.U.$ | 2 |
| $\gamma_\Omega$ | $N.U.$ | 0.003 |
| $\alpha$ | $N.U.$ | 0.005 |
| $\kappa$ | $g^{-1}$ | 0.05 |
| $\gamma_E$ | $kcal.g^{-1}.min^{-1}$ | 0.14 |
| $\eta$ | $min^{-1}$ | 0.00012 |
| $\rho_{FFM}$ | $kcal.g^{-1}$ | 1.8 |
| $\rho_{FM}$ | $kcal.g^{-1}$ | 9.4 |
| $\xi$ | $kcal$ | 400 |

**Table III.2** – Parameter units and default values used in the simulations. Parameter values have been chosen in order to characterize bistability. $N.U.$ denotes "non-dimensional unit".

where parameters $\rho_{FFM}$ and $\rho_{FM}$ denote the caloric densities of fat-free mass and fat mass respectively. Energy intake corresponds to the caloric content of food intake $FI$ characterized by the caloric density $\gamma_E$ of the food. Following (A2), energy expenditure is assumed to be proportional to fat mass and fat-free mass with a basal energy expenditure $\xi$ and a rate of energy expenditure $\eta$ [Jacquier et al., 2014; Nelson et al., 1992]. The energy balance is then defined as

$$EI - EE = \gamma_E FI - \eta(\rho_{FFM} FFM + \rho_{FM} FM + \xi). \tag{III.3}$$

The function $\Omega$ in (III.1)-(III.2) denotes the body composition function, describing the relationship between fat mass and fat-free mass. The expression of $\Omega$ has been deduced from experimental data [Guo and Hall, 2009, 2011], and is given by

$$\Omega := \frac{\mathrm{d}FFM}{\mathrm{d}FM} = \gamma_\Omega(1 + \alpha \exp(\kappa FM)), \tag{III.4}$$

characterized by parameters $\gamma_\Omega$, $\alpha$ and $\kappa$.

Fat-free mass $FFM$ can be explicitly obtained from fat mass $FM$ and initial conditions, by using (III.4). One obtains

$$FFM = \frac{\gamma_\Omega(\kappa FM + \alpha \exp(\kappa FM))}{\kappa} + C, \tag{III.5}$$

with

$$C := FFM(0) - \gamma_\Omega \left( FM(0) + \frac{\alpha}{\kappa} \exp(\kappa FM(0)) \right).$$

Hence, from (III.3), $EI - EE$ can be expressed as a function of $FI$ and $FM$ only, as follows

$$EI - EE = \gamma_E FI - \eta((\rho_{FM} + \rho_{FFM}\gamma_\Omega)FM + \frac{\rho_{FFM}\gamma_\Omega\alpha}{\kappa} \exp(\kappa FM) + \rho_{FFM}C + \xi).$$

Plasma leptin $L$ (in $ng.mL^{-1}$) is produced by adipocytes proportionally to fat mass [Jacquier et al., 2014; Tam et al., 2009] (Assumption (A3)), so

$$\frac{\mathrm{d}L}{\mathrm{d}t} = \gamma_L FM - \delta_L L, \tag{III.6}$$

where $\gamma_L$ is the rate of leptin production and $\delta_L$ the rate of leptin degradation (via renal elimination [Jacquier et al., 2014; Tam et al., 2009]).

Let denote by $R$ ($mol.L^{-1}$) the density of leptin receptors located in the hypothalamus, which mediate the inhibition of food intake by leptin. Leptin receptors expression is regulated by leptin [Martin et al., 2000; Myers et al., 2008; Pal and Sahu, 2003; Scarpace et al., 2005; Widdowson et al., 1997; Wilsey and Scarpace, 2004; Zhang et al., 1997] (Assumption (A4)). We assume that both production and degradation of $R$ are increased by leptin $L$, and we account for basal production ($\gamma_R$) and degradation ($\delta_R$) rates. Thus, the number of receptors will at first increase with leptin, then decrease when the concentration of leptin is high. It must be noted however that positive regulation of leptin receptor production by leptin is not necessary to obtain the results presented in Section III.3. The density of receptors then evolves according to the following equation,

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \gamma_R(1 + \lambda_{R1}L) - \delta_R(1 + \lambda_{R2}L^2)R. \tag{III.7}$$

Parameters $\lambda_{R1}$ and $\lambda_{R2}$ characterize the effect of leptin on the production ($\lambda_{R1}$) and degradation ($\lambda_{R2}$) of leptin receptors. Our main assumption is that the influence of leptin on degradation is more important than on production for high leptin concentrations. This assumption is satisfied, for instance, in the absence of positive regulation of receptor production (when $\lambda_{R1} = 0$), as soon as the degradation rate of receptors is an increasing

function of leptin concentration, which is in agreement with the literature [Martin et al., 2000; Zhang et al., 1997]. One may note that other leptin-dependent functions could be used for the description of production and degradation of receptors, provided that they satisfy the previously mentioned assumption. Our choice has been motivated by the will to obtain a simple, in terms of parameter number and dynamics, yet general model. It has been inspired by Topp et al. [Topp et al., 2000], who used a similar function to model the dynamics of $\beta$-cell mass.

Activation of leptin receptors in the hypothalamus leads to a pathway controlling food intake $FI$ (in grams) [Myers et al., 2008]. The response $\Phi_R(L)$ of the activation of leptin receptors can be described in terms of occupation theory [Kenakin, 2004; Ruffolo, 1982; Stephenson, 1956] by a Hill function (Assumption (A5)), where the maximal response is proportional to the density of receptors, given by

$$\Phi_R(L) = \frac{\phi R L^n}{L^n + \theta^n}, \tag{III.8}$$

with $\phi R$ the maximal response, $\theta$ a threshold corresponding to 50% of activation and the integer $n \geq 1$ a sensitivity coefficient.

Finally according to Assumption (A6), we assume food intake $FI$ is inhibited by the activation of leptin receptors [Friedman and Halaas, 1998; Myers et al., 2008], so

$$\frac{\mathrm{d}FI}{\mathrm{d}t} = \frac{\gamma_{FI}}{1 + \Phi_R(L)} - \delta_{FI} FI, \tag{III.9}$$

where $\gamma_{FI}$ describes the rate of stimulation of food intake, and $\delta_{FI}$ denotes an inhibition rate of food intake.

The system formed with equations (III.1), (III.6), (III.7), (III.8) and (III.9) describes the interactions between food intake and fat mass mediated by leptin and leptin receptors dynamics (see Figure III.2 for a state variable flow diagram). In the following, we focus on the dynamics of this system of equations, given by:

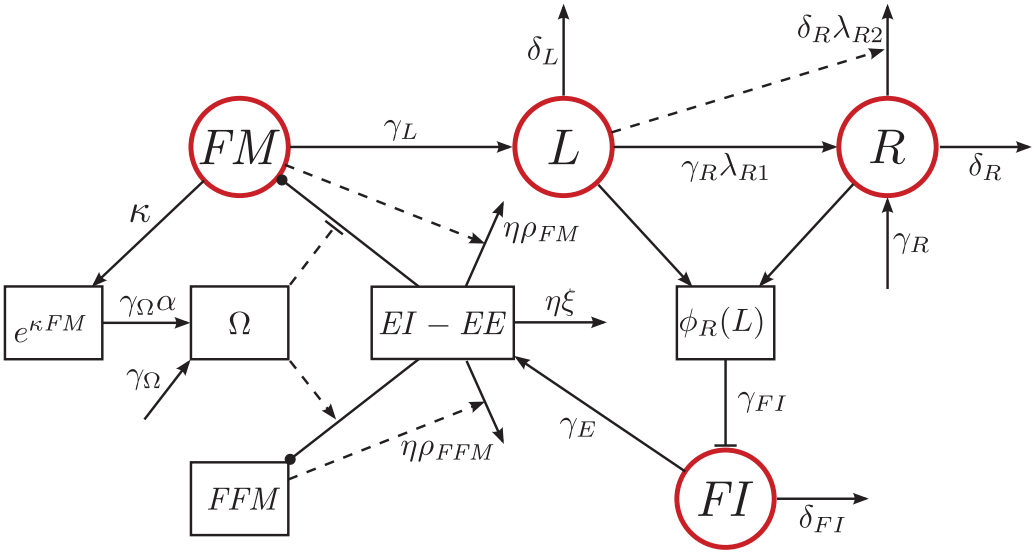


**Figure III.2** – State variable flow diagram of System (III.10). Main variables are displayed in red circles. Arrows indicate a positive contribution, bar-headed lines a negative contribution and dot-ended lines indicate a contribution that can be either positive or negative depending on the value of the considered quantity (here, only $EI - EE$, the energy balance, can positively contribute to fat mass and fat-free mass in some conditions, and negatively in other conditions). Straight lines represent either production or degradation of variables, while dashed lines represent the influence of one variable on a biological process.

$$\begin{cases} \dfrac{\mathrm{d}FM}{\mathrm{d}t} &= \dfrac{\gamma_E FI}{\rho_{FFM}\gamma_\Omega(1+\alpha\exp(\kappa FM))+\rho_{FM}} \\ & \quad -\dfrac{\eta((\rho_{FM}+\rho_{FFM}\gamma_\Omega)FM+\frac{\rho_{FFM}\gamma_\Omega\alpha}{\kappa}\exp(\kappa FM)+\rho_{FFM}C+\xi)}{\rho_{FFM}\gamma_\Omega(1+\alpha\exp(\kappa FM))+\rho_{FM}}, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L FM-\delta_L L, \\ \dfrac{\mathrm{d}R}{\mathrm{d}t} &= \gamma_R(1+\lambda_{R1}L)-\delta_R(1+\lambda_{R2}L^2)R, \\ \dfrac{\mathrm{d}FI}{\mathrm{d}t} &= \dfrac{\gamma_{FI}(L^n+\theta^n)}{L^n(1+\phi R)+\theta^n}-\delta_{FI}FI. \end{cases} \tag{III.10}$$

Before presenting in the next section how we use this model to investigate the development of leptin resistance, let us briefly comment on the non-negativity of the solutions of System (III.10).

Variables $L$, $R$ and $FI$ remain non-negative as long as the other variables involved in System (III.10) are positive, as expected. Solutions of the fat mass equation however can

become negative under specific conditions. Basically, if $FM = 0$ then $dFM/dt > 0$ if and only if

$$\gamma_E FI > \eta(\rho_{FFM}(\frac{\gamma_\Omega \alpha}{\kappa} + C) + \xi).$$

Consequently, when food intake $FI$ is close to zero and fat mass is also close to zero then the difference between energy intake and energy expenditure can be negative and solutions can become negative. This is a property of the Guo and Hall's model [Guo and Hall, 2009, 2011], which has been proposed to model fat and fat-free mass dynamics in either normal or obese states, associated with normal or high food intakes, but cannot account for an extreme situation corresponding to low food intake associated with low fat mass.

In order to ensure non-negativity of the solutions of System (III.10), we considered throughout this manuscript parameter values and initial conditions such that $EI - EE$ is positive for low fat mass, satisfying the assumptions in Guo and Hall [Guo and Hall, 2009, 2011] and hence do not induce a loss of positivity for $FM$.

System (III.10) will be used in Section III.3 to investigate the development of leptin resistance and how it can be related to obesity, based on the variation of food intake stimulation as detailed hereafter.

### III.2.2 Varying food consumption

In the previous sections, we considered all parameter values to be constant; however, the characteristics of a biological system evolve with time for a single individual. Parameters describing rates of creation and degradation (for example parameters $\delta_L$ and $\gamma_L$ in Equation (III.6)) or sensitivity, such as $\lambda_{R1}$, are impacted by aging. Environmental conditions can also induce variations, for example environment can impact food consumption. We choose here to focus only on the variations in parameter $\gamma_{FI}$, representing the stimulation rate of food intake, and we investigate how variations in $\gamma_{FI}$ can induce first the development of leptin resistance and then obesity. Variability in parameter value also exists between individuals, explaining why some individuals are more susceptible than others to develop obesity even if they are submitted to similar changes.

To model progressive changes in food intake, we temporally modify the parameter $\gamma_{FI}$ in the following way

$$\gamma_{FI} = \gamma_{FI}(t) = \gamma_{FI}^0 + g(t),$$

where $\gamma_{FI}^0$ represents the initial value of $\gamma_{FI}$ and $g(t)$ a temporal perturbation leading to an increase or a decrease in $\gamma_{FI}$. We assume that the modifications of food intake are negligible at short time scale (minutes, hours) and only impact the dynamics of the system after a long time (weeks, months). Indeed, modifications in food intake must be sustained for at least a few days to induce important metabolic modifications [Jacquier et al., 2014].

System (III.10) is either monostable or bistable depending on parameter values (see Section III.3.1). When the system is bistable, we can define a healthy state corresponding to the equilibrium with low fat mass and an obese state corresponding to high fat mass. Without perturbations, the solutions of System (III.10) remain close to the healthy equilibrium. As the system's equilibria depend on parameter values, changes of equilibria values and bifurcations may occur when varying $\gamma_{FI}$ (see Section III.3.1). As we are interested in pathways to leptin resistance and obesity, we assume in the following that initially System (III.10) is close to the healthy equilibrium, so initial conditions of $FM$, $L$, $R$ and $FI$ are close to the healthy steady state values. Without perturbation, the system remains in this state.

We consider different patterns of food intake, characterized by different functions $g(t)$ (see Figure III.3): a combination of two sigmoids (one increasing and the second one decreasing) and a sinusoid. These variations of $\gamma_{FI}$ induce a bypass of the regulation of food intake by leptin. We assume that this variations represent behavioral phenomena to regulate food intake and body weight.

The combination of two sigmoid functions corresponds to an increase in $\gamma_{FI}$ and then a decrease to a value lower (or higher) than the initial value. It is described by $g(t)$ with

$$g(t) = \begin{cases} 0, & \text{if } 0 < t < t_1, \\ \Delta_{\gamma_{FI}^1} \frac{2t^3 - 3(t_1+t_2)t^2 + 6t_1t_2t + t_1^2(t_1-3t_2)}{(t_1-t_2)^3}, & \text{if } t_1 < t < t_2, \\ \Delta_{\gamma_{FI}^1}, & \text{if } t_2 < t < t_3, \\ \Delta_{\gamma_{FI}^1} + \Delta_{\gamma_{FI}^2} \frac{-2t^3 + 3(t_3+t_4)t^2 - 6t_3t_4t - t_3^3 + 3t_3^2t_4}{(t_3-t_4)^3}, & \text{if } t_3 < t < t_4, \\ \Delta_{\gamma_{FI}^1} - \Delta_{\gamma_{FI}^2}, & \text{if } t > t_4, \end{cases} \tag{III.11}$$

where $\Delta_{\gamma_{FI}^1}$ is the amplitude of variation of the increasing sigmoid and $\Delta_{\gamma_{FI}^2}$ the amplitude of variation of the decreasing sigmoid. Parameters $t_1$ and $t_2$ define the increasing part of the function while $t_3$ and $t_4$ delimit the decreasing part. The plateau phase, associated with a high food intake stimulation equal to $\gamma_{FI}^1$, has a duration given by $t_3 - t_2$. The final value $\gamma_{FI}^2$ resulting from the two variations can be higher or lower than the initial

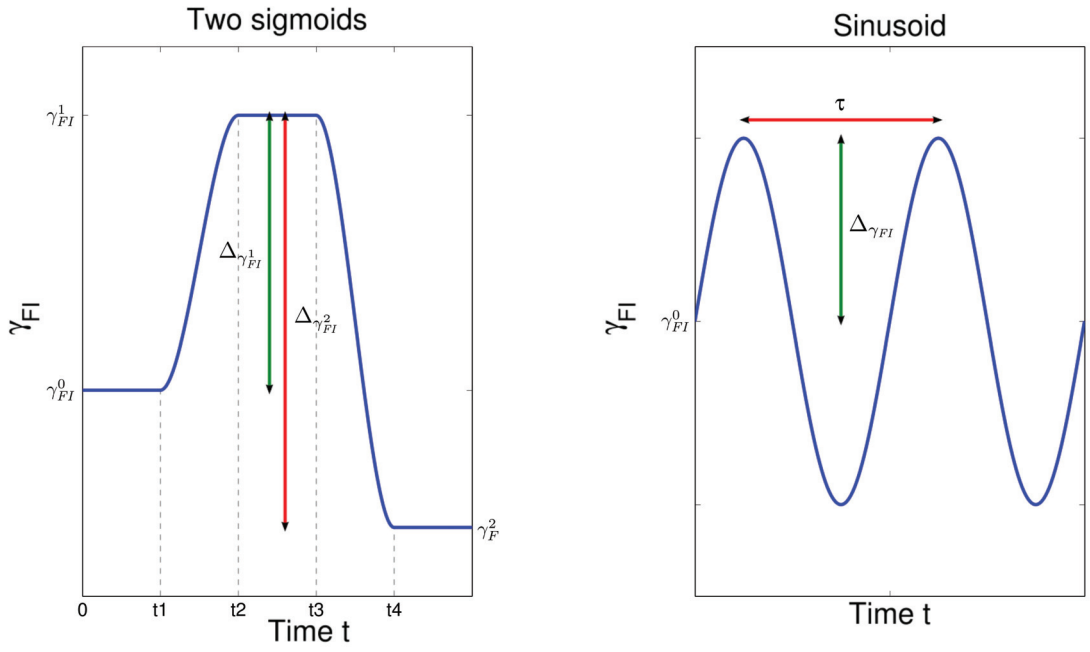


**Figure III.3** – Functions used to temporally modify the stimulation rate of food intake ($\gamma_{FI}$): a double sigmoid (left) and a sinusoid (right).

value $\gamma_{FI}^0$. This $\gamma_{FI}$ function represents a progressive increase in food consumption, which will stabilize at some point. The decreasing part can be the consequence of reduction of food intake, such as a diet to reverse the effects of the initial increase.

The sinusoid function is defined by

$$g(t) = \Delta_{\gamma_{FI}} \sin\left(\frac{2\pi}{\tau}t\right), \tag{III.12}$$

with $\Delta_{\gamma_{FI}}$ representing half the amplitude of variation of the function and $\tau$ the period of the sinusoid. This function describes regular increase and decrease of food intake stimulation with a period of $\tau$ minutes and can model a repetition of the double sigmoid function. It can simulate conscious repeated attempts to limit food intake after an increase. For small periods, it can represent day to day compensations in food intake.

## III.3 Results

### III.3.1 Existence of equilibria and stability analysis

Equilibria of System (III.10) correspond to constant solutions. Only the positive solutions are hereafter considered to be physiologically relevant.

We can deduce from System (III.10) that an equilibrium $(FM^*, L^*, R^*, FI^*)$ satisfies the conditions

$$L^* = \frac{\gamma_L}{\delta_L} FM^*, \tag{III.13}$$

$$R^* = \frac{\gamma_R(1+\lambda_{R1}L^*)}{\delta_R(1+\lambda_{R2}L^{*2})}, \tag{III.14}$$

$$FI^* = \frac{\gamma_{FI}(L^{*n}+\theta^n)}{\delta_{FI}(L^{*n}(1+\phi R^*)+\theta^n)}, \tag{III.15}$$

and

$$FI^* = \frac{\eta}{\gamma_E}\left(\frac{\rho_{FFM}\gamma_\Omega\alpha}{\kappa}\exp(\kappa FM^*) + (\rho_{FM}+\rho_{FFM}\gamma_\Omega)FM^* + \rho_{FFM}C + \xi\right). \tag{III.16}$$

Using (III.13) and (III.14), the expressions (III.15) and (III.16) for $FI^*$ can be written as functions of $FM^*$. We define

$$f_1(FM) := \frac{\gamma_{FI}\delta_R(\delta_L^2+\lambda_{R2}\gamma_L^2FM^2)(\gamma_L^nFM^n+\delta_L^n\theta^n)}{\delta_{FI}(aFM^{n+2}+bFM^{n+1}+cFM^n+dFM^2+e)}$$

with $a = \gamma_L^{n+2}\delta_R\lambda_{R2}$, $b = \gamma_L^{n+1}\phi\delta_L\gamma_R\lambda_{R1}$, $c = \gamma_L^n\delta_L^2(\delta_R+\phi\gamma_R)$, $d = \delta_L^n\theta^n\delta_R\gamma_L^2$ and $e = \delta_L^{n+2}\delta_R\theta^n$,

and

$$f_2(FM) := \frac{\eta}{\gamma_E}\left(\frac{\rho_{FFM}\gamma_\Omega\alpha}{\kappa}\exp(\kappa FM) + (\rho_{FM}+\rho_{FFM}\gamma_\Omega)FM + \rho_{FFM}C + \xi\right).$$

An intersection between $f_1$ and $f_2$ defines a value $FM^*$, and consequently an equilibrium of System (III.10). Positive equilibria exist if

$$f_1(0) > f_2(0),$$

as $f_2(FM)$ is strictly increasing and $f_1$ admits an upper bound for $FM = 0$. Therefore the system must satisfy the following condition:

$$\frac{\gamma_{FI}}{\delta_{FI}} > \frac{\eta}{\gamma_E}\left(\frac{\rho_{FFM}\gamma_\Omega\alpha}{\kappa} + \rho_{FFM}C + \xi\right).$$

This condition displays a relationship between food intake (represented by the ratio $\gamma_{FI}/\delta_{FI}$) and energy expenditure. In order to obtain positive equilibria, the energy balance $EI - EE$ must be positive for low fat mass (one may note that the same condition ensures positivity of the solutions of System (III.10), see the end of Section III.3.1). The number of equilibria is then equal to one or three depending on the parameter values (see Figure III.4 for examples). A detailed analysis of a simplified model is presented in A.

The equilibrium with low leptin concentration and low fat mass (see Figures III.4.B and III.4.C) corresponds to a healthy state while the equilibrium with high leptin concentration and high fat mass corresponds to an obese state with leptin resistance. In addition, the healthy state has a high number of receptors whereas the obese state has a low number of receptors. One may note that fat free mass is also increased in the obese state, compared to the healthy state, as creation of fat mass leads to creation of some fat free mass, yet it has been observed experimentally that the increase in fat-free mass is smaller than the increase in fat mass [Hall, 2007] and the same is obtained with the set of parameter values in Table III.2.

To study the stability of equilibria, we determine the Jacobian matrix $J$ of System (III.10) at a given equilibrium $(FM^*, L^*, R^*, FI^*)$, given by

$$J = \begin{pmatrix} J_{LL} & 0 & 0 & J_{ML} \\ J_{LR} & J_{RR} & 0 & 0 \\ J_{LI} & J_{RI} & J_{II} & 0 \\ 0 & 0 & J_{IM} & J_{MM} \end{pmatrix},$$

with:

$$\begin{aligned}
J_{LL} &= -\delta_L < 0, & J_{ML} &= \gamma_L > 0, \\
J_{RR} &= -\delta_R(1 + \lambda_{R2}L^{*2}) < 0, & J_{LR} &= \gamma_R\lambda_{R1} - 2\delta_R\lambda_{R2}L^*R^*, \\
J_{II} &= -\delta_{FI} < 0, & J_{LI} &= \frac{-n\gamma_{FI}\theta^n\phi L^{*n-1}R^*}{(L^{*n}(1+\phi R^*) + \theta^n)^2} < 0, \\
J_{RI} &= \frac{-\phi\gamma_{FI}L^{*n}(L^{*n} + \theta^n)}{(L^{*n}(1+\phi R^*) + \theta^n)^2} < 0, & J_{IM} &= \frac{\gamma_E}{\rho_{FFM}\gamma_\Omega(1 + \alpha\exp(\kappa FM^*)) + \rho_{FM}} > 0,
\end{aligned}$$

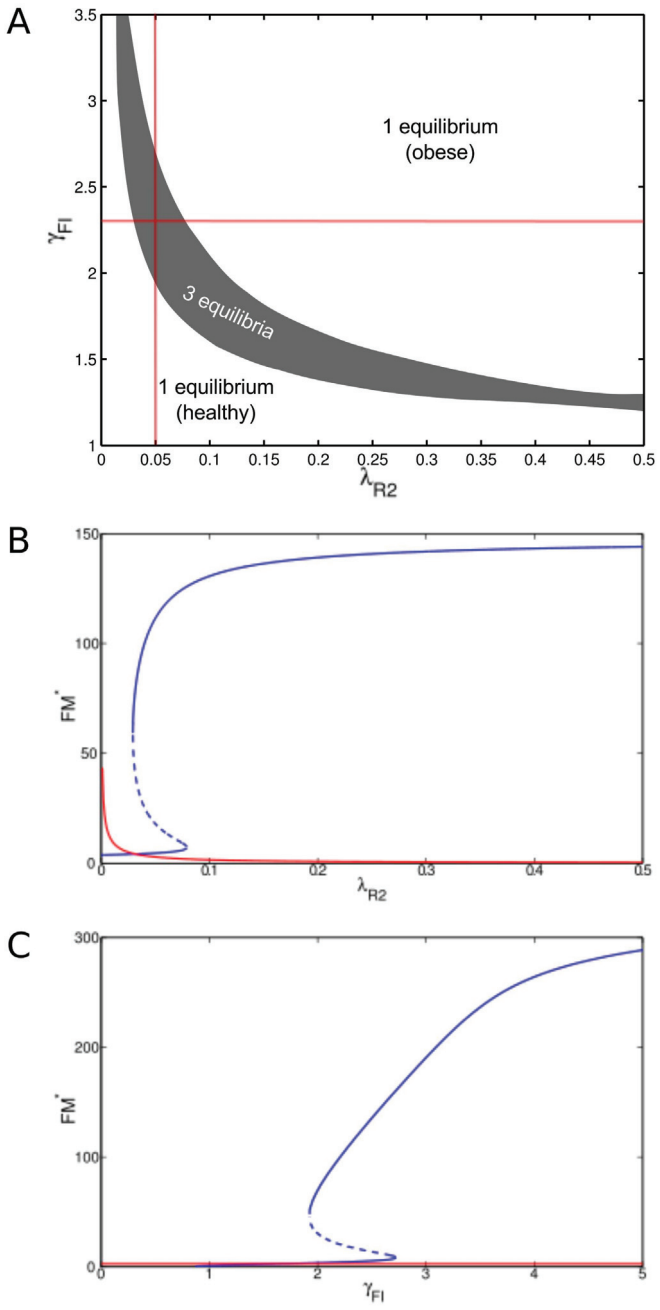


**Figure III.4** – A. Diagram displaying the existence of equilibria in the $(\gamma_{FI}, \lambda_{R2})$-plane (other parameter values are fixed, see Table III.2). When increasing parameter values, the system encounters bifurcations with hysteresis. The red lines indicate the sections displayed in B and C. B. Bifurcation diagram for $\lambda_{R2}$, characterizing the influence of leptin on receptors degradation, with $\gamma_{FI} = 2.3\ g.min^{-1}$ and all other parameter values given by Table III.2. C. Bifurcation diagram for $\gamma_{FI}$, representing the stimulation rate of food intake, with $\lambda_{R2} = 0.05\ mL^2.ng^{-2}$ and all other parameter values given by Table III.2. The system displays between one and three equilibria and a hysteresis when increasing the parameter value. Solid blue lines indicate stable equilibria, whereas dashed blue lines indicate unstable equilibria. Stable equilibria fulfill conditions (III.18), with in particular $z > 0$. The value of $\tilde{FM}$, defined in (III.17), is displayed as a red line.

and, using (III.16),

$$J_{MM} = -\eta < 0.$$

The only coefficient with a non constant sign is $J_{LR}$, and $J_{LR}$ is positive when

$$L^* < \tilde{L} := \frac{-\lambda_{R2} + \sqrt{\lambda_{R1}^2 \lambda_{R2} + \lambda_{R2}^2}}{\lambda_{R1}\lambda_{R2}},$$

or equivalently

$$FM^* < F\tilde{M} := \frac{\delta_L}{\gamma_L}\tilde{L}. \tag{III.17}$$

The characteristic polynomial $P$ is then defined as

$$P(\chi) = \chi^4 + u\chi^3 + v\chi^2 + w\chi + z, \qquad \chi \in \mathbb{C},$$

with:

$$\begin{aligned}
u &= -J_{II} - J_{LL} - J_{RR} - J_{MM} > 0,\\
v &= J_{II}J_{LL} + J_{II}J_{RR} + J_{II}J_{MM} + J_{LL}J_{RR} + J_{LL}J_{MM} + J_{RR}J_{MM} > 0,\\
w &= -J_{II}J_{LL}J_{RR} - J_{II}J_{LL}J_{MM} - J_{II}J_{RR}J_{MM} - J_{LL}J_{RR}J_{MM} - J_{IM}J_{LI}J_{ML} > 0,\\
z &= J_{II}J_{LL}J_{RR}J_{MM} + J_{IM}J_{LI}J_{RR}J_{ML} - J_{IM}J_{LR}J_{RI}J_{ML}.
\end{aligned}$$

The sign of $z$ changes depending on parameter and equilibrium values. In particular, if $FM^* \leq F\tilde{M}$, then both $J_{LR}$ and $z$ are positive.

If real parts of the roots of the characteristic polynomial are negative, the associated equilibrium is locally asymptotically stable while if at least one root has a positive real part, the equilibrium is unstable.

The Routh-Hurwith Criterion, applied to $P$, allows us to conclude that all the roots of $P(\chi)$ are negative or have a negative real part if and only if the following conditions are satisfied,

$$\begin{cases} z > 0, \\ uv > w, \\ w(uv - w) > u^2 z. \end{cases} \tag{III.18}$$

For a given set of parameter values, the system displays 3 equilibria, an unstable one

between 2 stable ones, with a hysteresis (see Figure III.4.A and A for a detailed stability analysis of a simplified system). It is easy to numerically determine the equilibria values and the area of stability (see Figures III.4.B and III.4.C for examples of $\lambda_{R2}$ and $\gamma_{FI}$ dependent stability areas). With the parameter values used for Figures III.4.B and III.4.C, conditions $uv > w$ and $w(uv - w) > u^2 z$ are satisfied as soon as $z > 0$, and the stability is consequently determined only by the sign of $z$. In particular, for values of $FM^*$ lower than $\tilde{FM}$, $z$ is positive and the equilibrium is stable.

It must be noted that, for instance, when $\lambda_{R1} = 0$ (absence of positive regulation of leptin receptor production by leptin), bistability also occurs, event though $J_{LR} < 0$: the condition $J_{LR} > 0$ is not a necessary and sufficient condition for bistability, because $z$ can be positive even though $J_{LR}$ is negative.

System (III.10) can then be bistable or monostable, in this latter case it can either be stable around a healthy or an obese equilibrium. The development of leptin resistance and obesity can only occur if the solution reaches the basin of attraction of the obese equilibrium. Dynamically going from one equilibrium to the other one can only be achieved by perturbing the parameter values influencing the existence of equilibria and the size of the basins of attraction.

### III.3.2 Constant leptin infusion can lead to leptin resistance

Leptin resistance is characterized by the inability of the system to integrate leptin signals. Constant injections of leptin in healthy rats showed that after an initial phase of efficient regulation of food intake a second phase, occurring after a few days, corresponding to the development of leptin resistance, was associated with high food intake and high leptin levels [Pal and Sahu, 2003; Sahu, 2002].

We first assume that body weight remains constant, with both fat mass and fat-free mass constant, and we model a constant injection of leptin. Let denote by $\Lambda$ ($ng.mL^{-1}.min^{-1}$) the leptin injection, then Equation (III.6) can be written

$$\frac{\mathrm{d}L}{\mathrm{d}t} = \gamma_L FM + \Lambda - \delta_L L, \tag{III.19}$$

with $FM$ constant.

Starting close to the healthy equilibrium (for variables $L$, $R$ and $FI$, whereas $FM$ is

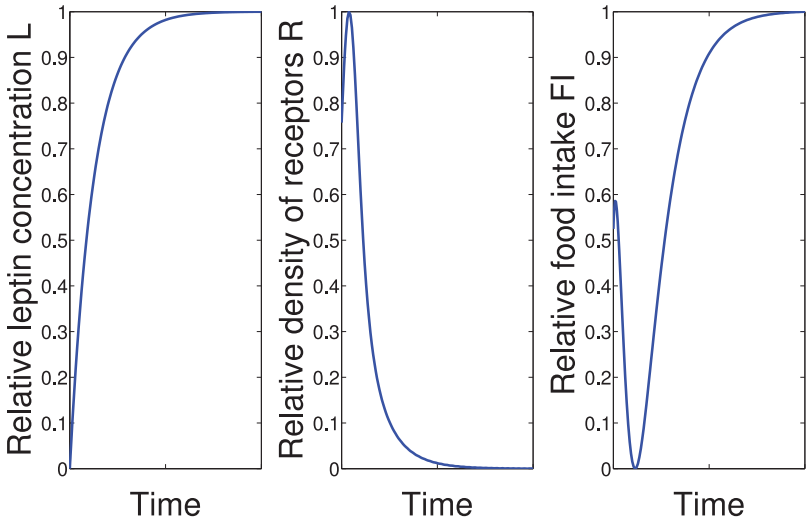


**Figure III.5** – Relative values of leptin, leptin receptors and food intake (normalized between 0 and 1, for illustration purpose, using the following formula: $(x(t) - min(x(t)))/(max(x(t)) - min(x(t)))$, where $x = R, L, FI$), following a constant leptin injection. The initial value is close to the healthy equilibrium.

constant), we observe in Figure III.5:

- first, an increase in leptin level, an increase in the density of receptors, and a decrease in food intake, corresponding to a healthy behavior;
- then, a higher leptin level, a decrease of the density of leptin receptors to low levels, and an increase in food intake which stabilizes at a value higher than the initial value.

This situation is characteristic of leptin resistance. Depending on the intensity of the injection, results quantitatively change, yet they are qualitatively equivalent.

If the starting point is close to the obese equilibrium, the system is already leptin resistant. Leptin injection in that case will have no impact on the dynamics of the system (results not shown). Also in this case, depending on the initial value and the strength of the injection, one can observe a slight decrease in the density of receptors and a slight increase in food intake: the system is becoming more leptin resistant.

We now use the full model (System (III.10)) with leptin dynamics given by (III.19) to illustrate the appearance of leptin resistance. We use experimental data from Pal and Sahu [Pal and Sahu, 2003], describing food intake and body weight dynamics in rats during a constant injection of leptin. Results are presented in Figure III.6. In addition to simulation results for food intake and body weight dynamics (Figures III.6.A and III.6.B)

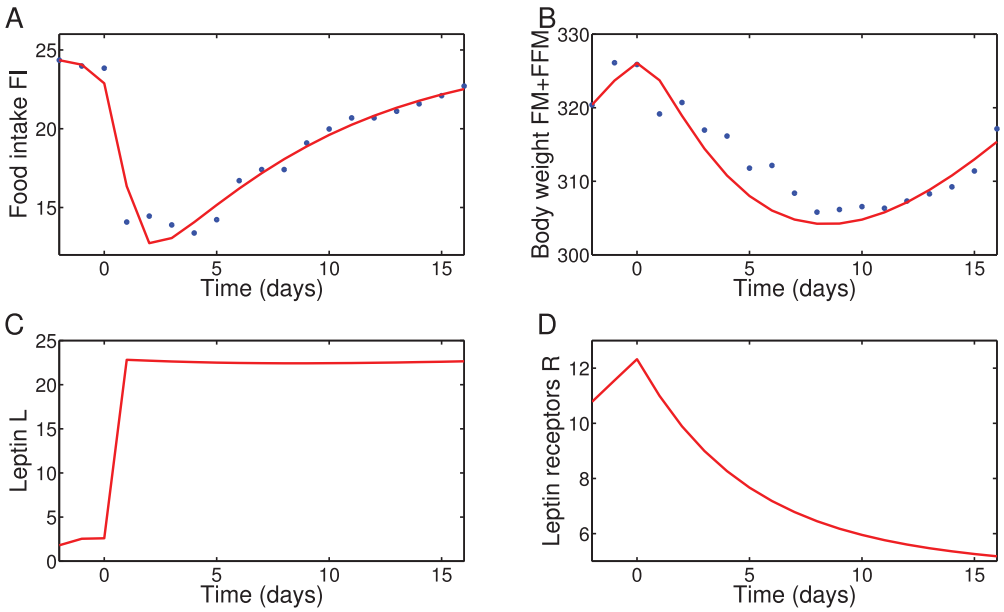


**Figure III.6** – Simulation results (red lines) compared to [Pal and Sahu, 2003] data (blue dots representing the mean value at each time). Experimental data have been obtained by infusing leptin for 16 days directly into the brain of Sprague-Dawley rats. Constant injection of leptin starts at time 0 and lasts until the end of the experiment (day 16). Parameter values are taken from Jacquier et al. [Jacquier et al., 2014] or estimated (see Table III.3). A. Food intake dynamics. Food intake drops at day 0, then stays low for a few days and increases to its initial level. B. Body weight dynamics. Body weight starts to decrease when leptin injection starts and then increases from day 10. C. Leptin concentration. It becomes and remains high following the injection, totally saturating leptin receptors and inducing a downregulation of the receptors. D. Leptin receptors. They increase previous to the leptin injection, then continuously decrease until the end of the experiment. The system is progressively becoming leptin resistant.

we also present simulated leptin dynamics and leptin receptor dynamics (Figure III.6.C and III.6.D). The constant leptin injection starts at day 0, and previously the system is in a healthy state. Following leptin injection one observes a strong decrease in food intake which increases again after 3-4 days, and a slower decrease of body weight which increases again from day 10. The model correctly reproduces these dynamics (Figures III.6.A and III.6.B). In addition, due to a constant leptin injection from day 0 the leptin concentration quickly reaches a plateau and saturates throughout the experiment, while the number of leptin receptors continuously decreases towards low levels (Figures III.6.C and III.6.D). This situation characterizes leptin resistance.

| Parameter | Unit | Value | Reference |
|---|---|---|---|
| $\gamma_L$ | $ng.g^{-1}.min^{-1}$ | 0.0954 | derived |
| $\delta_L$ | $min^{-1}$ | 0.074 | Zeng et al. [1997] |
| $\gamma_R$ | $mol.L^{-1}.min^{-1}$ | $5.87 \times 10^{-4}$ | derived |
| $\delta_R$ | $min^{-1}$ | $3.26 \times 10^{-6}$ | derived |
| $\lambda_{R1}$ | $ng^{-1}$ | $1.8 \times 10^{-4}$ | derived |
| $\lambda_{R2}$ | $ng^{-2}$ | $1.94 \times 10^{-4}$ | derived |
| $\delta_{FI}$ | $min^{-1}$ | $1.19 \times 10^{-3}$ | derived |
| $\gamma_{FI}$ | $kcal.min^{-1}$ | $3.46 \times 10^{-4}$ | derived |
| $\phi$ | $L.mol^{-1}$ | 1 | derived |
| $\theta$ | $ng.mL^{-1}$ | 57.22 | derived |
| $n$ | $N.U.$ | 2 | derived |
| $\gamma_\Omega$ | $N.U.$ | 2.2 | Jacquier et al. [2014] |
| $\alpha$ | $N.U.$ | $7.27 \times 10^{-10}$ | Jacquier et al. [2014] |
| $\kappa$ | $g^{-1}$ | 0.269 | Jacquier et al. [2014] |
| $\gamma_E$ | $min^{-1}$ | 1 | derived |
| $\eta$ | $min^{-1}$ | $1.77 \times 10^{-5}$ | derived |
| $\rho_{FFM}$ | $kcal.g^{-1}$ | 9.4 | Guo and Hall [2009, 2011] |
| $\rho_{FM}$ | $kcal.g^{-1}$ | 1.8 | Guo and Hall [2009, 2011] |
| $\xi$ | $kcal$ | 1413.6 | derived |
| $\Lambda$ | $ng.min^{-1}$ | 30 | derived |

**Table III.3** – Parameter units and values used to generate simulated dynamics from System (III.10) compared with data from [Pal and Sahu, 2003], and presented in Figure III.6. *N.U.* denotes "non-dimensional unit", when the value is taken from the literature, the corresponding reference is indicated.

### III.3.3 Varying the stimulation rate of food intake can induce leptin resistance and obesity

We showed in the previous section that our model is able to characterize the development of leptin resistance. We are now going to theoretically investigate its ability to describe pathways to leptin resistance and obesity in order to make predictions that would be testable experimentally.

We are particularly interested in the influence of progressive variations in food intake on the development of leptin resistance and obesity. Leptin resistance is characterized by a high concentration of leptin $L$ which is not associated with a decrease in fat mass $FM$. Obesity corresponds in this model to a state of the system with increased fat mass $FM$. Variations in food intake are indeed influenced by variations in the stimulation rate of food intake, represented by the parameter $\gamma_{FI}$ in (III.9). Variations in the rate of inhibition ($\delta_{FI}$) can be realistically neglected.

We consider 2 scenarii corresponding to 2 different ways of varying food intake. The parameter $\gamma_{FI}$ is assumed to be given by

$$\gamma_{FI} = \gamma_{FI}(t) = \gamma_{FI}^0 + g(t),$$

where $\gamma_{FI}^0$ represents a basal value of food intake stimulation (which depends on individual characteristics, so variations in $\gamma_{FI}^0$ account for inter-individual variability), and $g(t)$ describes a time-dependent modification of food intake habits. We consider either increasing then decreasing (double sigmoid) variations or oscillating (sinusoidal) variations (see Figure III.3 and Section III.2.2). Variations in the value of $\gamma_{FI}$ induce modifications in equilibria values and stability. In the bistable case, a solution of System (III.10) cannot go from one equilibrium to the other, so the only way to develop leptin resistance and obesity starting from a healthy state is that, due to some perturbation, the healthy stable equilibrium no longer exists at some point.

#### III.3.3.1 Increasing then decreasing food intake stimulation rate

We study the effect of progressive changes in food intake on the development of leptin resistance and obesity. These changes are represented by an increase of the stimulation rate $\gamma_{FI}$, followed by a stabilization and later a decrease. We assume that $\gamma_{FI}^0$ corresponds either to the bistable system or the system with only the monostable healthy equilibrium and that the initial condition of System (III.10) is close to the healthy equilibrium.

The initial increase between $\gamma_{FI}^0$ and $\gamma_{FI}^1 = \gamma_{FI}^0 + \Delta_{\gamma_{FI}^1}$ leads to an increase in body weight. The importance of the increase depends on the amplitude $\Delta_{\gamma_{FI}^1}$ and the duration $t_2 - t_1$ (see (III.11)). If $\gamma_{FI}^1$ is in the bistable area, the solution remains close to the healthy equilibrium. Then the increase in fat mass is limited to normal physiological variations, without development of leptin resistance and obesity. Yet, with a plateau value $\gamma_{FI}^1$ corresponding to a monostable obese equilibrium the fat mass keeps increasing and the solution reaches the basin of attraction of the obese equilibrium, characterized by high fat mass and leptin levels (see Figure III.7.A). This represents a progressive pathway to leptin resistance and obesity, where a progressive increase in food consumption has almost no impact for some time but can lead to leptin resistance and obesity if the increase does not stop.

The same variation characterized by $\Delta_{\gamma_{FI}^1}$ and $\Delta_{\gamma_{FI}^2}$ has different consequences depending on the initial value $\gamma_{FI}^0$ (see Figure III.8). Thus, a fixed or $\gamma_{FI}^0$-dependent variation will lead

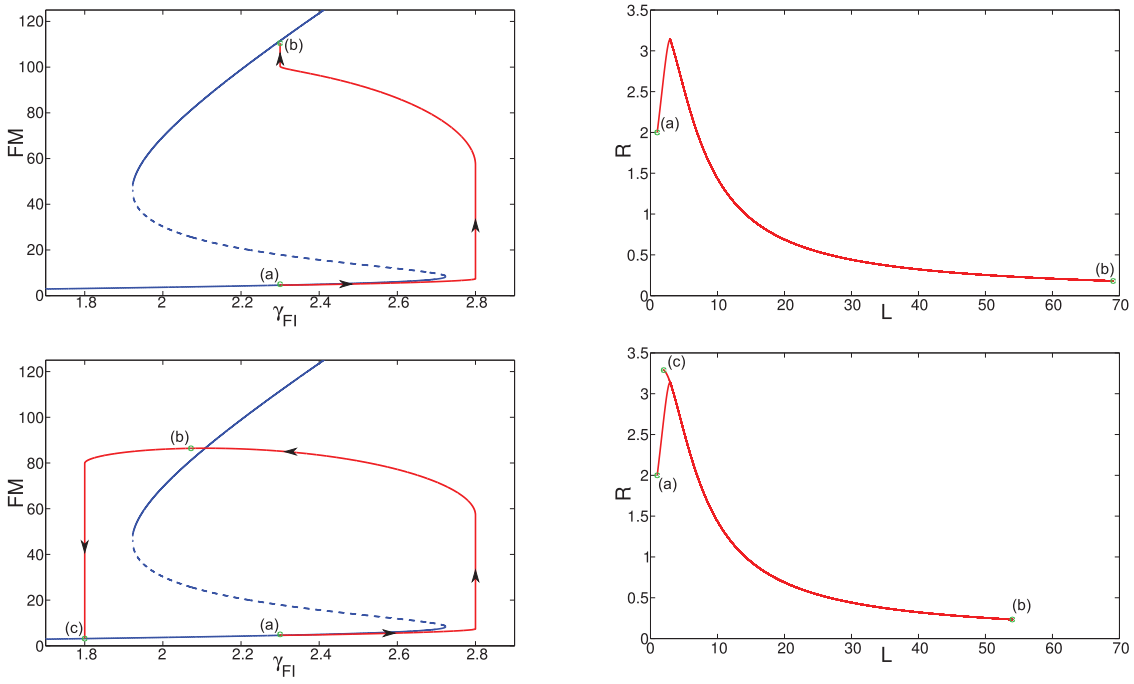


**Figure III.7** – Evolution of the value of $FM$ (in red) with the values of the equilibrium $FM^*$ (in blue) on the left column, and evolution of the values of $L$ and $R$ on the right column, for an increasing then decreasing sigmoid-like function $\gamma_{FI}$. The initial condition of System (III.10), denoted by (a), is close to the healthy equilibrium and $\gamma_{FI}^0$ is located in the bistable area. A. For $\gamma_{FI}$ increasing from 2.3 to 2.8 and then going back to 2.3, the solution goes from the healthy equilibrium (a) to the obese equilibrium (b). The system progressively becomes leptin resistant, with low density of receptors and high concentration of leptin at the end of the variation. B. For $\gamma_{FI}$ increasing from 2.3 to 2.8 and then decreasing to 1.8, the solution reaches the obese equilibrium before going back to the healthy equilibrium. The system, initially healthy (a), becomes leptin resistant (b), and then returns to the healthy state (c) when $\gamma_{FI}$ reaches its final value. This evolution follows an hysteresis cycle.

to different values of the system after some time. Depending on the value $\gamma_{FI}^0$, the system either stays in the healthy state or becomes obese and leptin resistant. Indeed inter-individual variability has an important impact on the development of leptin resistance and obesity.

Assuming that $\gamma_{FI}^1$ is located in the monostable obese area and $\gamma_{FI}^0$ in the bistable area, we study the effect of a decrease, occurring after the plateau phase, of the stimulation rate $\gamma_{FI}$ on the solution. The behavior of the solution depends on the amplitude $\Delta_{\gamma_{FI}^2}$ of the decrease. If $\Delta_{\gamma_{FI}^2} \leq \Delta_{\gamma_{FI}^1}$, corresponding to a final stimulation rate $\gamma_{FI}^2$ higher or equal to the initial value, the solution remains in the basin of attraction of the obese equilibrium (see Figure III.7.A). This situation corresponds to the case where an individual progressively increases their food intake, then becomes leptin resistant and obese but cannot go back to their initial state (healthy) even by reducing food intake to the original

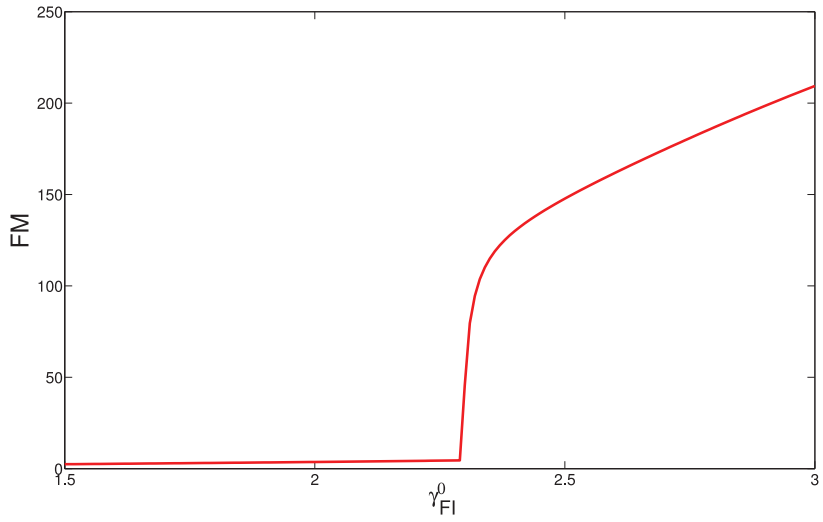


**Figure III.8** – Final value of the fat mass $FM$ after an increase of the food intake stimulation $\gamma_{FI}$, followed by a decrease (sigmoid-like function, see Figure III.3 left). The initial condition of System (III.10) is close to the healthy equilibrium. The initial value of $\gamma^0_{FI}$ ranges from 1.5 to 3 and the amplitude of the variation depends on the initial value ($\Delta_{\gamma^1_{FI}} = \Delta_{\gamma^2_{FI}} = 0.2\gamma^0_{FI}$).

level. One may note that this situation can also occur for $\Delta_{\gamma^2_{FI}} > \Delta_{\gamma^1_{FI}}$, if $\gamma^2_{FI}$ corresponds to the bistable area.

In order for the solution to go back to the healthy equilibrium, the decrease in the stimulation rate must be more important than the increase and the final value $\gamma^2_{FI}$ must correspond to the monostable healthy equilibrium. The solution then follows an hysteresis cycle when increasing and then decreasing (see Figure III.7.B). In order to return to the healthy state from the obese state, the food intake stimulation must be sustained at a lower value than it was at the beginning, for a time long enough.

One may note that if the plateau time (between $t_2$ and $t_3$) is too short, the solution may not reach the basin of attraction of the obese equilibrium and just varies around the healthy equilibrium. Short-time increases of food intake stimulation rate do not significantly impact body weight and can be easily compensated, which is not the case for a sustained increase.

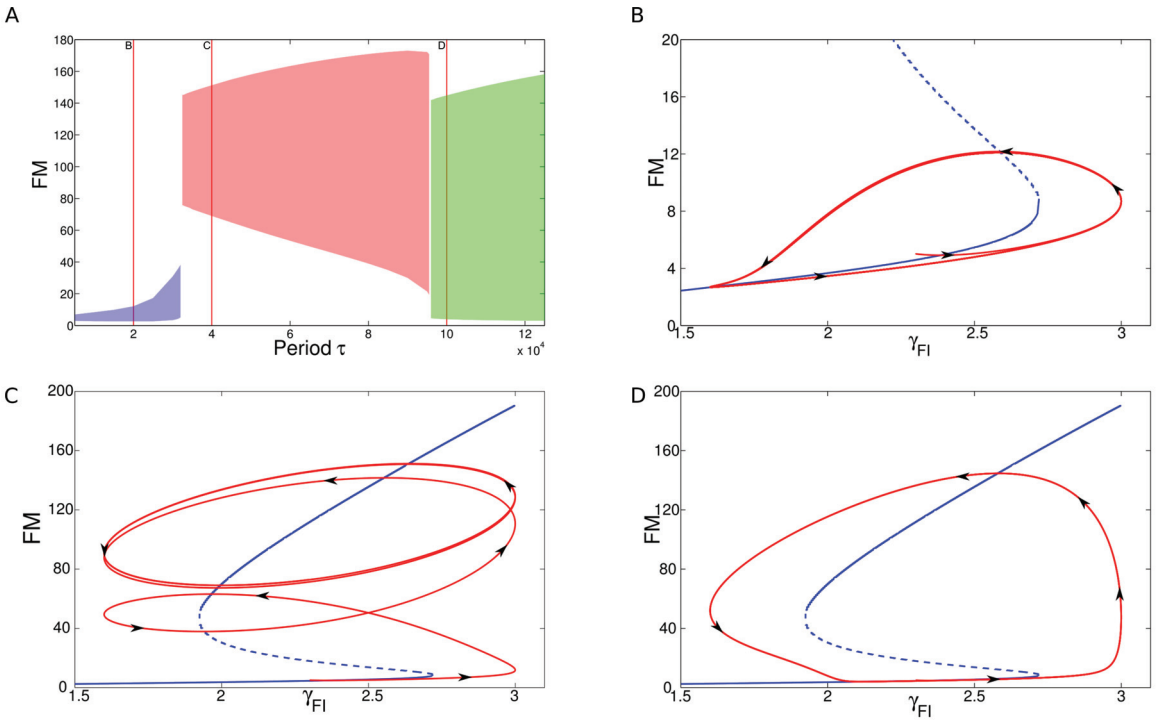


**Figure III.9** – Evolution of fat mass $FM$ when the stimulation rate of food intake $\gamma_{FI}$ oscillates between 1.6 and 3 as a sine function. The initial condition of System (III.10) is close to the healthy equilibrium and $\gamma_{FI}^0 = 2.3$. A. Evolution of the amplitude of the sustained oscillations of $FM$ (colored areas) for $\gamma_{FI}$ oscillating with an increasing period $\tau$. The amplitude $\Delta_{\gamma_{FI}}$ remains the same. Three areas corresponding to different amplitudes of $FM$ are observed: for a low period $\tau$ (in blue), for an intermediate period (in red) and for a high period (in green). The period of oscillations corresponding to figures B, C and D is displayed by vertical red lines. B-C-D. Evolution of the value of $FM$ (in red) and of the value of the equilibrium $FM^*$ (in blue) as a function of $\gamma_{FI}$. B. For a low period of oscillations, the solution oscillates around the healthy equilibrium. The system is not leptin resistant (low leptin level, high density of receptors). C. The period of oscillations is doubled and the solution oscillates around the obese equilibrium. The solution reaches a limit cycle where the system is in a leptin resistant state (low density of receptors and high concentration of leptin). D. For a period of oscillations equal to 5 times the period of Figure A, the solution oscillates between the healthy and the obese equilibrium. The system oscillates between a state of leptin resistance and a healthy state.

### III.3.3.2 Oscillating food intake stimulation

We study the impact of repeated increases and decreases in food intake stimulation on the development of leptin resistance and obesity. These variations are modeled as a sine function centered on $\gamma_{FI}^0$, as described in (III.12). We assume that $\gamma_{FI}^0$ corresponds either to the bistable system or the system with only the monostable healthy equilibrium, and that the initial condition of System (III.10) is close to the healthy equilibrium. One may note that varying $\gamma_{FI}$ to follow a sine function leads to oscillations in variable values at the same frequency as $\gamma_{FI}(t)$. Moreover, depending on the initial value $\gamma_{FI}^0$, the amplitude

and the period of oscillations, the behavior of the system will follow different patterns. In the following, we describe these different cases.

We first consider the case with $\gamma_{FI}$ varying only in the bistable area. The solution oscillates around the equilibrium value closer to the initial condition of System (III.10) (obese or healthy, results not shown). This can represent day to day variations observed in most biological systems and that have almost no impact on the long-term body weight.

We assume now that the variations of $\gamma_{FI}$ cover the entire bistable area and parts of the monostable areas both on the left and right sides of the bistable area. We also assume that $\gamma_{FI}^0$ is located close to the center of the bistable area, in order to have the same time spent in both monostable areas. Though, the solution should have the same possibility to join the basin of attraction of the remaining equilibrium in both monostable areas. The period of oscillations also has an impact on the behavior of the system. For example, the amplitude of the variation in fat mass is a function of the period of the oscillations (see Figure III.9.A). If the period of oscillations is low, the solution oscillates around the healthy equilibrium, even if, for some values of $\gamma_{FI}$, the healthy equilibrium does not exist anymore (see Figure III.9.B). As the period of oscillations is low, the oscillations can model short term variations in food intake, that have only a limited impact on body weight dynamics [Chow and Hall, 2014]. Increasing the period of oscillations leads to changes in the dynamics of the solution, which can leave the healthy equilibrium to oscillate around the obese equilibrium (see Figure III.9.C). In that case, the body weight is trapped around the obese state and it is not possible for the individual to leave the obese state, despite a periodic reduction in food intake. The individual is also leptin resistant, with a low density of receptors and a high concentration of leptin. If the period of oscillations is large enough, the solution oscillates between the two stable equilibria (see Figure III.9.D). This latter case could correspond to a yo-yo effect observed in individuals who progressively gain weight before following a strict diet and repeat the process several times. One may notice that increasing the amplitude of oscillations eases the change of equilibrium: the period of oscillations needed to leave the healthy equilibrium is reduced. From a biological point of view, if a healthy individual increases their food intake in an important way, it should be easier and quicker to reach an obese state with leptin resistance than slightly increasing food intake. A similar phenomenon is also observed when going from the obese state to the healthy state by decreasing food intake.

If $\gamma_{FI}$ ranges in the bistable area and in the monostable obese equilibrium area only, the behavior of the solution is similar to the previous case (results not shown) except that it is not possible to go back to the healthy equilibrium. For a given amplitude of oscillations

and an initial condition for the System (III.10) close to the healthy equilibrium, a low period of oscillations corresponds to oscillations around the healthy equilibrium. If the period of oscillations is increased, the solution can reach the basin of attraction of the obese equilibrium when the system is monostable and then oscillates around the obese equilibrium.

When the solution oscillates around the obese equilibrium without being able to go back to the healthy equilibrium (see for instance Figure III.9.C), the only possibility to go back to the healthy state from the obese state is to apply a different perturbation to the system. This perturbation can be applied to any parameter of the system and should allow the system to reach the monostable healthy area for a time long enough for the solution to join and remain in the basin of attraction of the healthy equilibrium.

## III.4 Discussion

Leptin resistance is observed in humans and in rodents, and is characterized by the inability of the body to respond to high concentrations of leptin in the blood, which should normally induce a downregulation of food intake. Mechanisms behind the development of this resistance are not fully known. Obesity, associated with high amounts of fat and leptin in the body, is a cause and a consequence of leptin resistance [Zhang and Scarpace, 2006].

In this work, we developed a mathematical model of body weight and food intake dynamics, considering a regulation mediated only by the leptin/leptin receptors system. It is noticeable that regulatory mechanisms have been inspired by experimental observations in rodents and previous models of body weight dynamics for rodents. Although describing a simplified reality, this system has 2 stable equilibria (depending of course on parameter values) associated to a healthy state (no leptin resistance and low fat mass) and a leptin-resistant/obese state (high fat mass and high leptin levels). At a constant healthy fat mass, a constant leptin infusion induces a state of leptin resistance, characterized by an increased leptin concentration, a reduced density of receptors and an increased food intake. We showed that our model was able to correctly reproduce the dynamics of body weight and food intake during leptin injection that leads to development of leptin resistance, using data from Pal and Sahu [Pal and Sahu, 2003]. We then showed that the system can dynamically go from the healthy state to the leptin resistant one, and described potential pathways to obesity. The underlying mechanism relies on leptin's up

and down regulation of its own receptors. High leptin concentration strongly down regulates leptin receptors – by increasing degradation rate – whereas low leptin concentration has the opposite effect. Under this assumption, we showed that it is possible to become leptin resistant and obese, starting from a healthy state, by progressively increasing food intake stimulation rate in order to ignore leptin signals.

We also investigated the potential consequences of a sinusoidal variation of food intake stimulation and showed that it could theoretically lead to leptin resistance and obesity under some conditions on the period and the amplitude of oscillations. Thus, low amplitude and low period oscillations have no impact on the transition from a healthy state to a leptin resistant state. Increasing the period and/or the amplitude of the oscillation increases the probability for the system to become leptin resistant and obese. The extreme case with high amplitude and high period leads to an alternation of the system between healthiness and leptin resistance, which is considered to be totally reversible in our model. This behavior is qualitatively in agreement with the biology, since leptin resistance is considered to be reversible or at least partially reversible. Introducing variability in the initial parameter values leads to different behaviors of the system, which has a different susceptibility to develop leptin resistance and obesity when submitted to the same perturbation. If the food intake stimulation is high, the probability to develop leptin resistance and obesity after a perturbation is more important than for a low value. To our knowledge, the hysteresis cycle obtained when varying parameters values has not been observed experimentally. It may be possible to observe it by monitoring body weight, food intake, leptin concentration and leptin receptors expression when progressively changing the caloric content of food intake over a long time scale in rodents.

One may think about other ways of inducing leptin resistance and obesity. Instead of associating the development of leptin resistance to a temporal modification of one or several parameter values (here, the food intake stimulation rate) a stochastic modification of food intake could lead to leptin resistance. Our attempts to induce leptin resistance and obesity by adding a Wiener process to Equation (III.9), describing food intake dynamics, did not provide relevant results (results not shown): only large amounts of noise were shown to induce a modification of the system, switching from a healthy state to a leptin-resistant state, and they do not appear biologically realistic. It would be more reasonable to consider that stochastic events combined with a temporal modification of some characteristics of the system (as described above) could lead to leptin resistance, consequently they could not be considered as the main cause of leptin resistance.

Another hypothesis for leptin resistance development could be a delay in the integration

of leptin signals. For instance, production and/or degradation of leptin receptors may not be instantaneously modified by leptin levels, or food intake stimulation may not react to the current state of the leptin/leptin receptors system. We tested the assumption of a delayed response of food intake regulation to changes in receptor density, yet this does not allow to describe the development of leptin resistance (results not shown): adding a delay in System (III.10) can destabilize either one or both equilibria, yet the destabilization is associated with the appearance of oscillating solutions, but not with a pathway to leptin resistance. At this stage, a preliminary conclusion would be that a delay can strengthen a leptin resistant situation, but cannot induce leptin resistance.

It is noticeable that our model only includes one type of leptin receptors, located in the hypothalamus, that induce a regulation of food intake. There exist experimental evidences that leptin resistance can also occur at the blood-brain barrier, leading to a reduced ratio between blood leptin and plasma leptin and to an inability of the system to respond to intravenous injections of leptin. It is possible to improve the model by including the transport of leptin from the blood to the brain via its receptors and observe different types of leptin resistance. This is left for a future work, as experimental measurements would be needed to better characterize leptin resistances. It may also be mentioned that other regulators of food intake and body weight exist [Morton et al., 2006, 2014; Schwartz et al., 2000], such as adaptation of the energy expenditure and the effect of hormones other than leptin, which were not considered here. However, the behavior of our model is qualitatively relevant from a biological point of view. This model, consisting in only 4 differential equations, efficiently describes the mechanisms of leptin resistance (without assuming degradations due to aging), at least in rodents, and the development of obesity based on the regulation of leptin receptors density by leptin.

# Chapter IV

# A new and simplified model of body weight dynamics

## IV.1 Introduction

The regulation of body weight is based on the energy balance, which is in particular regulated by hormones and nutrients, as we have seen in details in Section I.1. The purpose of this regulation is presumably to limit important gain or loss of weight. Energy balance corresponds to the difference between energy intake and energy expenditure. The regulation of energy intake is performed by the regulation of food consumption by multiple factors, but also depends on external factors such as food composition or caloric content. Energy expenditure is composed of physical activity, basal metabolic rate and adaptive thermogenesis, each component can be modulated but within a limited range.

When modeling energy balance, assumptions have to be made to describe both energy intake and expenditure. Energy intake can be considered as proportional to food intake. Energy expenditure is more complex to estimate and is often considered as a linear or polynomial function of body composition and/or body weight [Livingston and Kohlstadt, 2005; Nelson et al., 1992]. When in addition, body weight is assumed to be separated into fat and fat-free mass, it is necessary to define a way to partition energy. In Chapter II and III, body weight is separated into fat mass and fat-free mass (which are linked by a specific function) and energy expenditure, which depends linearly on fat and fat-free mass, includes a constant term representing the basal metabolic rate. This description of energy expenditure can lead to positivity issues (see Sections III.2.1 and IV.2.1), so,

in this chapter, we propose a modification of the description of body weight dynamics which preserves body weight dynamics, including fat mass and fat-free mass dynamics, and ensures positive solutions (in particular positive body weights).

First, the simplification of the equation describing the dynamics of body weight is presented, as well as the description of fat mass from body weight necessary to consider the dynamics of leptin. This simplified equation is then integrated within the two previous models (Chapters II and III) to replace the equations describing fat mass and fat-free mass dynamics. The newly obtained systems are analyzed and their results are compared to experimental data and to the results of the original models to test the relevance of the simplification. A full model of the regulation of food intake, body weight and energy expenditure is then introduced as a combination of the two new and simplified systems to take into account the regulation of food intake and body weight by leptin, leptin receptors, ghrelin and glucose.

## IV.2 New model of body weight dynamics

In this section I present the equations developed to simplify the previously described models (Chapters II and III) and analyze the resulting systems. I will compare the new models to the previous models to test the relevance of the simplification in the following sections (IV.3 and IV.4). This simplification will eventually be introduced in a new model combining both models in order to have a more complete description of the dynamics of body weight and food intake, including regulation by hormones and able to describe the development of leptin resistance.

### IV.2.1 Description

The model of body weight dynamics previously described in Chapter II (Equations (II.1) and (II.2)) and used in Chapter III (Equations (III.1) and (III.2)) displays some analysis and positivity issues. The positivity of the solution is not maintained in some cases (see Figure IV.1), for example when fat mass is close to 0 it is possible that fat mass becomes negative if the food intake is not important enough to compensate energy expenditure (as detailed in Section III.2.1). The main cause of this positivity issue is the definition of energy expenditure in the equations describing fat mass and fat-free mass (originally

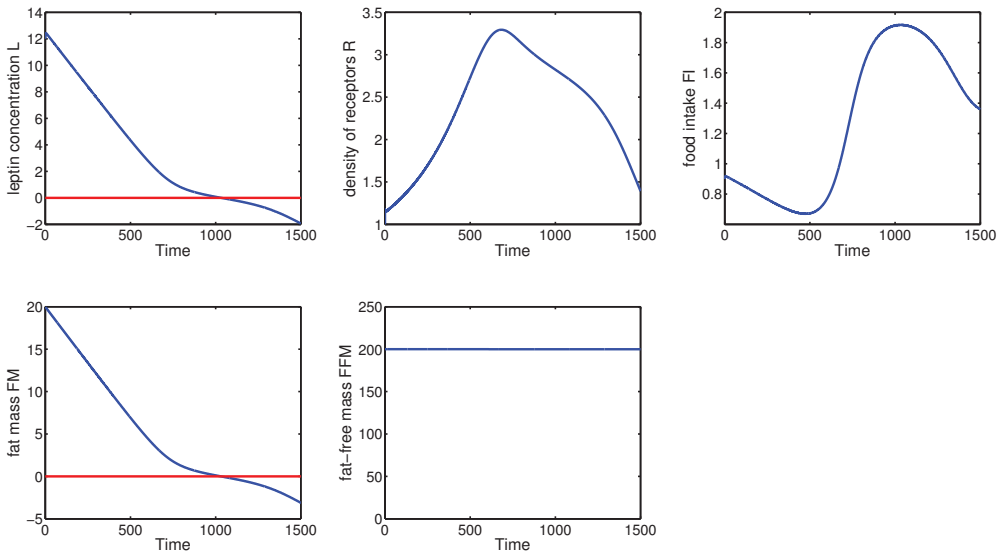


**Figure IV.1** – Example of a simulation of System (III.10) with all parameter values and initial conditions positive but with fat mass and leptin becoming negative. All parameter values are taken from Table III.2, except $\eta = 0.0004$ instead of $\eta = 0.00012$.

adapted from [Guo and Hall, 2009, 2011]). Energy expenditure is assumed to be proportional to fat mass and fat-free mass, and to have a constant component, thus allowing to remove more energy than what is available in the body. However, the model is not intended to describe extreme cases with the fat mass totally depleted. In these cases, the formula used to describe energy expenditure is not valid anymore, as other phenomena appear to induce a more important reduction in energy expenditure. If the mean food intake is not too low, fat mass should not decrease too much and the solution should remain positive.

To improve the properties of the systems developed in Chapters II and III, we introduce a new equation describing body weight dynamics, similarly to [Chow and Hall, 2008], by combining and simplifying the equations describing fat mass and fat-free mass from [Guo and Hall, 2009, 2011]. As we have seen in Chapter II, the variations of fat mass and fat-free mass depend on the energy balance (equal to the difference between energy intake and energy expenditure). Energy intake is still assumed to be proportional to food intake, which was already the case for the previous equations. We assume that energy expenditure is now proportional to body weight, without any distinction between fat and fat-free mass. This leads to the equation describing body weight $M$ (grams), equal to the

sum of fat mass and fat-free mass, as follows

$$\frac{\mathrm{d}M}{\mathrm{d}t} = \gamma_M F - \delta_M M, \tag{IV.1}$$

with $F$ the food intake (g), $\gamma_M$ the absorption of the food ($\mathrm{min}^{-1}$) and $\delta_M$ the rate of energy expenditure ($\mathrm{min}^{-1}$). We will see later that the properties of the system are preserved by the simplification of body weight dynamics.

In order to describe leptin dynamics, which is produced proportionally to fat mass, we need a way to estimate fat mass from total body weight. We assume that fat mass is proportional to body weight, with a proportion coefficient $k$ depending on body weight, so $k = k(M)$: the larger the body weight, the larger $k$. The function $k(M)$ is an increasing bounded function, with values ranging between 0 and $k_{max} < 1$, which is the maximal percentage of fat mass. One may note that $k_{max}$ has to be strictly lower than 1, due to the fat-free component of body weight which is never equal to 0.

Hence, the equation for leptin becomes

$$\frac{\mathrm{d}L}{\mathrm{d}t} = \gamma_L k(M) M - \delta_L L. \tag{IV.2}$$

These two equations can be included in both previous models, with conservation of the predictive aspects of the models, as described in the following sections. One can immediately note that these equations preserve the positivity of the solutions without restrictions on parameter values (except that they all have to be non-negative), unlike what was obtained in Section III.2.1, for the model using the description of body weight dynamics from [Guo and Hall, 2009, 2011].

### IV.2.2 Estimation of fat mass from total body weight

As Equation (IV.1) describes body weight without distinction between fat mass and fat-free mass, it is necessary to determine the best function $k(M)$ describing the percentage of fat mass from body weight, in order to deduce fat mass and fat-free mass (and the relationship between them) from body weight and to correctly describe leptin production. The estimation of $k(M)$ is specific to the population considered in the data set and is performed by fitting multiple functions to experimental data, such as polynomial functions of degree $n$, rational algebraic fractions, logarithmic functions and exponential functions.

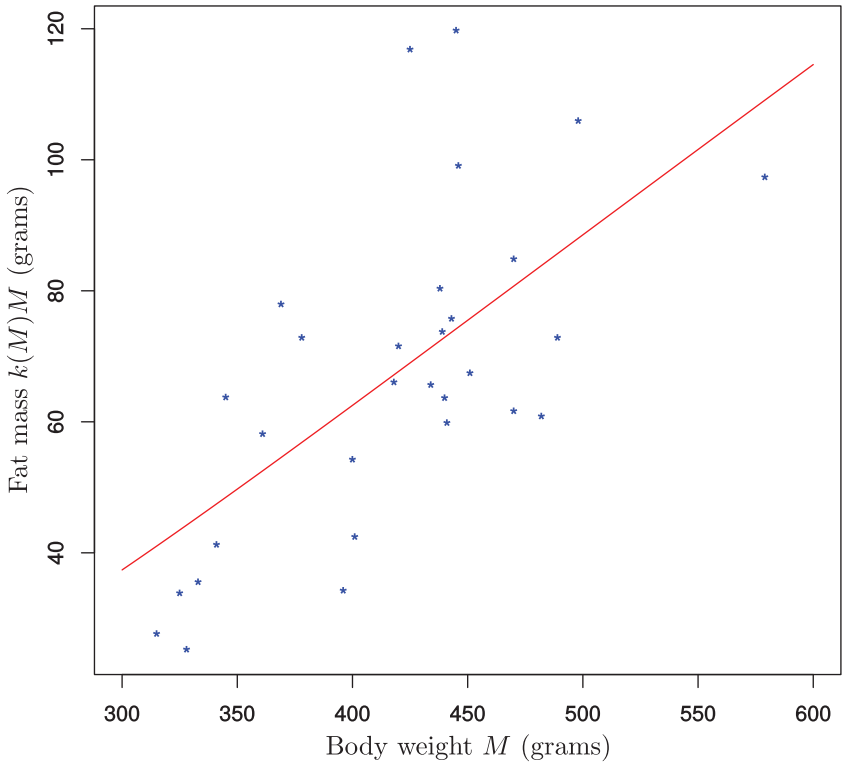


**Figure IV.2** – Evolution of fat mass (in grams) as a function of body weight (in grams) in Wistar rats. The function $k(M) = aM^2/(M^2+b)$ is fitted to experimental data from [Jacquier et al., 2014], with $a = 23.2\%$ and $b = 77523$ g$^2$.

The best function to predict fat mass from body weight is then selected from the tested functions, using Akaike information criterion to maximize the accuracy of the prediction while minimizing the number of parameter values to estimate. For example, for rodents, functions

$$k(M) = \frac{aM^n}{M^n + b}$$

give the best compromise between accuracy of the predictions and number of parameter values (here 3 parameter values characterize the function $k(M)$).

For rats, the data we used correspond to body weight and fat mass from [Jacquier et al., 2014]. This data set is composed of data from 5 groups of 6 Wistar rats: 3 groups submitted to a hypocaloric diet for 8 weeks with different patterns of food availability, 1 group submitted to an *Ad libitum* diet for 8 weeks and a control group sacrificed at day 0 (see Figure II.2). The following function was selected, corresponding to a compromise

between the number of parameters and the accuracy,

$$k(M) = \frac{aM^2}{M^2 + b} \tag{IV.3}$$

with $a = 23.2\%$ and $b = 77523$ g$^2$ (see Figure IV.2). In this case, $k_{max} = a$, parameter $a$ then corresponds to the maximum for $k(M)$ and $\sqrt{b} \simeq 278$ g, the value of $M$ for which $k(M) = a/2$. One can note that the variability between individuals can be important and that $k(M)$ is only providing a mean value, specific to the data set used for the parameter values estimation. This function will be used for the simulations throughout all this chapter.

In rats, the percentage of fat mass depends on the strain, age and sex of the animals. The characteristics of the food also impact the percentage of fat mass: most strains of rats submitted to a high-fat diet exhibit an important increase in their percentage of body fat [Schemmel et al., 1970]. Wistar rats fed with standard diet have a percentage of body fat (approximately 13% at 20 weeks of age for both males and females) lower than the estimated maximum fat percentage (23%) but it is not the case when they are fed with a high-fat diet (around 35% of body fat at 20 weeks) [Schemmel et al., 1970]. For the range of body weight considered latter in this chapter, the mean percentage of fat in the body should remain lower than the estimated maximum of 23% (approximately 17% of lipid for 600g Wistar rats [Newby et al., 1990]). The estimated value for $a$ is then in agreement with percentages observed in Wistar rats populations.

Once the function $k(M)$ is determined it is possible to include Equations (IV.1) and (IV.2) in the systems described in Chapters II and III and study them. This is what we do in Sections IV.3 and IV.4.

## IV.3 Hormonal regulation of body weight

The simplification described in the previous section is applied to the System (II.1)–(II.8) described in Chapter II, with equations for fat mass, fat-free mass and leptin replaced by Equations (IV.1) and (IV.2), with Equation (IV.3) describing the percentage of fat mass as a function of body weight. This system describes the dynamics of food intake and body weight, with hormonal regulations by ghrelin and leptin and adaptation of energy expenditure.

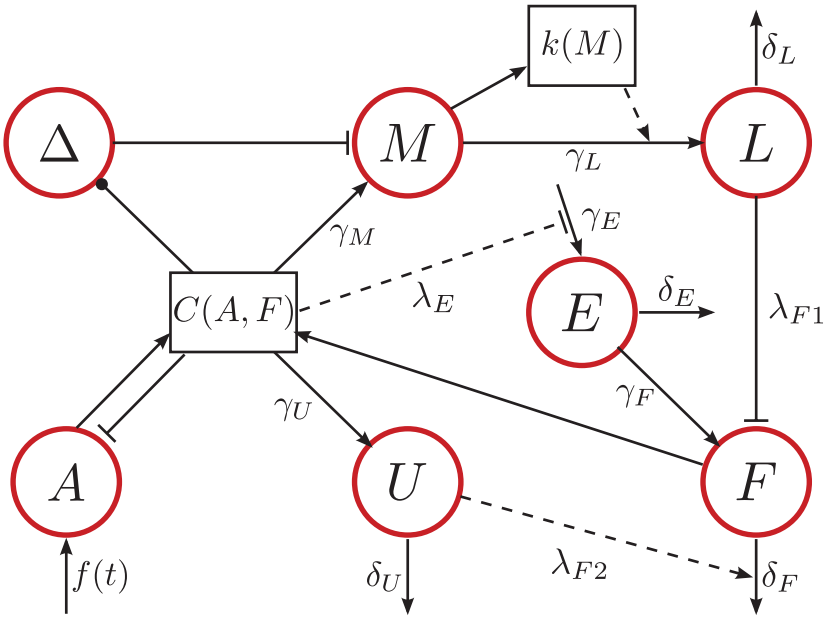


**Figure IV.3** – Variable flow diagram corresponding to System (IV.4). Bar-headed lines indicate an inhibition, straight arrows a production and dashed lines represent an influence on the degradation or the production. Lines ending with a dot indicate positive or negative influence (depending on the variable and parameter values).

### IV.3.1 Model

The system describing the hormonal regulation of food intake with adaptation of energy expenditure is defined as follows (see Figure IV.3),

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \gamma_M C(A,F) - \Delta M, \\ \dfrac{\mathrm{d}U}{\mathrm{d}t} &= \gamma_U C(A,F) - \delta_U U, \\ \dfrac{\mathrm{d}E}{\mathrm{d}t} &= \dfrac{\gamma_E}{1+\lambda_E C(A,F)} - \delta_E E, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L k(M) M - \delta_L L, \\ \dfrac{\mathrm{d}F}{\mathrm{d}t} &= \dfrac{\gamma_F E}{1+\lambda_{F1} L} - \delta_F(1+\lambda_{F2} U) F, \\ \dfrac{\mathrm{d}\Delta}{\mathrm{d}t} &= \epsilon \left( \dfrac{1}{\tau} \displaystyle\int_{t-\tau}^{t} C(A(v),F(v))\mathrm{d}v - \dfrac{1}{\tau'} \int_{t-\tau'}^{t} C(A(v),F(v))\mathrm{d}v \right), \\ \dfrac{\mathrm{d}A}{\mathrm{d}t} &= f(t) - C(A,F), \end{cases} \tag{IV.4}$$

with $M$ (g) the body weight, $U$ (g) the plasma glucose, $E$ (pg.mL$^{-1}$) the ghrelin concentration, $L$ (ng) the plasma leptin, $F$ (kcal) the expected food intake (denoted as hunger in Chapter II, it represents the food consumed if it is available *Ad libitum*). We denote by $A$ (kcal) the available food and $C(A,F)$ (kcal) the consumed food, depending on the expected food intake and the available food. In Chapter II, we used $C(A,F) = \min(A,F)$ to represent a consumption of food equal to its expected value or to the available food. The rate of energy expenditure $\Delta$ (min$^{-1}$) is considered as a variable instead of a fixed parameter in this model, it is then denoted as $\Delta$ instead of $\delta_M$. The function $f(t)$ represents the input of food as a function a time, but it can also represent a removal of food depending on the cases. Parameters correspond to the parameters presented in Chapter II.

### IV.3.2 Analysis

System (IV.4) is studied considering *Ad libitum* feeding, corresponding to $C(A,F) = F$ and no equation describing food availability.

An equilibrium $(M^*, U^*, E^*, L^*, F^*, \Delta^*)$ of System (IV.4) is determined by the following conditions:

$$\begin{cases} M^* &= \dfrac{\gamma_M F^*}{\Delta^*}, \\ U^* &= \dfrac{\gamma_U F^*}{\delta_U}, \\ E^* &= \dfrac{\gamma_E}{\delta_E(1+\lambda_E F^*)}, \\ L^* &= \dfrac{\gamma_L k(M^*) M^*}{\delta_L}, \\ F^* &= \dfrac{\gamma_F E^*}{\delta_F(1+\lambda_{F2}U^*)(1+\lambda_{F1}L^*)}, \\ \Delta^* &= \Delta_0, \end{cases}$$

with $\Delta_0$ the initial value for the rate of energy expenditure $\Delta$. An equilibrium then corresponds to the solutions of

$$\delta_F\delta_E F^*(1+\lambda_E F^*)\left(1+\lambda_{F2}\frac{\gamma_U F^*}{\delta_U}\right)\left(1+\lambda_{F1}\frac{\gamma_L k(\gamma_M F^*/\Delta_0)\gamma_M F^*}{\delta_L \Delta_0}\right) = \gamma_F\gamma_E. \quad \text{(IV.5)}$$

For $k(M) = aM^2/(M^2+b)$ (see Section IV.2), Equation (IV.5) is equivalent to

$$A_6F^{*6} + A_5F^{*5} + A_4F^{*4} + A_3F^{*3} + A_2F^{*2} + A_1F^* + A_0 = 0, \tag{IV.6}$$

with $A_i > 0$ for $i = 1, 3, 4, 5, 6$ and $A_0 < 0$. The system has one or three positive equilibria, depending on the sign of:

$$A_2 = b\left(\lambda_E + \lambda_{F2}\frac{\gamma_U}{\delta_U}\right) - \frac{\gamma_F}{\delta_F}\frac{\gamma_E}{\delta_E}\left(\frac{\gamma_M}{\Delta_0}\right)^2,$$

as follows,

— if $A_2 > 0$, Equation (IV.5) has only a single positive root, and System (IV.4) has a single positive equilibrium,

— if $A_2 < 0$, Equation (IV.5) has one or three positive roots, so the system has one or three positive equilibria.

It is then possible, by carefully choosing parameter values to have only a single positive equilibrium. One may note that the existence of equilibria does not depend on parameter $\lambda_{F1}$, which represent the sensitivity to leptin in the regulation of food intake, nor any parameter related to leptin ($\delta_L$ and $\gamma_L$). The existence of one or three positive equilibria highly depends on $\Delta_0$ and the choice of function $k(M)$. If $k(M)$ is a polynomial function, System (IV.4) has only a single positive equilibrium, since only coefficient $A_0$ in(IV.6) is negative, however, polynomial functions do not verify the condition $k(M) < 1$ for all $M > 0$ and do not predict well experimental data. If $k(M)$ is assumed to be constant, with $k(M) = \kappa < 1$, we can immediately deduce from Equation (IV.5) that the system has a single positive equilibrium. One can note that $k(M) = \kappa$ corresponds to the case $k(M) = aM^2/(M^2+b)$ with $b = 0$, leading to $A_2 < 0$, which, in this case, does not imply the existence of three positive roots, as $A_1 = 0$ when $b = 0$.

As $\Delta(t)$ can be deduced from $F(t)$ by the following expression,

$$\Delta(t) = \Delta_0 + \int_0^t \epsilon\left(\frac{1}{\tau}\int_{u-\tau}^{u} F(v)\mathrm{d}v - \frac{1}{\tau'}\int_{u-\tau'}^{u} F(v)\mathrm{d}v\right)\mathrm{d}u,$$

the stability analysis can be performed only on equations for $M$, $L$, $E$, $U$ and $F$.

The Jacobian matrix $J$ of System (IV.4), reduced to the equations for $M$, $U$, $E$, $L$ and

$F$, at a given equilibrium $(M^*, U^*, E^*, L^*, F^*, \Delta^*)$, is given by

$$J = \begin{pmatrix} -\Delta_0 & 0 & 0 & 0 & \gamma_M \\ 0 & -\delta_U & 0 & 0 & \gamma_U \\ 0 & 0 & -\delta_E & 0 & \frac{-\gamma_E \lambda_E}{(1+\lambda_E F^*)^2} \\ \frac{\gamma_L a M^{*2}(M^{*2}+3b)}{(M^{*2}+b)^2} & 0 & 0 & -\delta_L & 0 \\ 0 & -\delta_F \lambda_{F2} F^* & \frac{\gamma_F}{1+\lambda_{F1} L^*} & \frac{-\gamma_F \lambda_{F1} E^*}{(1+\lambda_{F1} L^*)^2} & -\delta_F(1+\lambda_{F2} U^*) \end{pmatrix}.$$

This matrix allows to calculate the characteristic polynomial, which has the following expression:

$$P(\chi) = \chi^5 + \alpha_4 \chi^4 + \alpha_3 \chi^3 + \alpha_2 \chi^2 + \alpha_1 \chi + \alpha_0,$$

with $\alpha_i > 0$ for all $i$. The characteristic polynomial has then no positive real root, but can have complex roots with positive real part. According to Routh-Hurwitz criteria, all roots of $P(\chi)$ are negative or have negative real parts and thus the equilibrium $(M^*, U^*, E^*, L^*, F^*, \Delta_0)$ is asymptotically stable if the following conditions are fulfilled:

— $\alpha_4 \alpha_3 \alpha_2 > \alpha_2^2 + \alpha_4^2 \alpha_1$,

— $(\alpha_4 \alpha_1 - \alpha_0)(\alpha_4 \alpha_3 \alpha_2 - \alpha_2^2 - \alpha_4^2 \alpha_1) > \alpha_0(\alpha_4 \alpha_3 - \alpha_2)^2 + \alpha_4 \alpha_0^2$.

One may note that System (IV.4) possess a delay differential equation that can modify the dynamics, by for example inducing oscillations around the unstable steady states. In the general case, it is not possible to determine analytically the behavior of the model, which will depend on the availability of food and the adaptation of the rate of energy expenditure. However, some special cases other than *Ad libitum* feeding, such as no food intake, can also be considered analytically. In any case, stability analysis of System (IV.4) can be performed numerically, based on the above-mentioned Routh-Hurwitz criteria.

### IV.3.3 Comparison to experimental data

In order to test the relevance of the model including the simplification, we compare its predictive aspect to the predictive aspect of the model from Chapter II, on two different data sets: experimental data presented in Chapter II and similar experimental data obtained during 16 weeks.

| Parameter | Value | Unit |
|---|---|---|
| $\gamma_M$ | 0.199 | $\text{g.kcal}^{-1}.\text{min}^{-1}$ |
| $\gamma_F$ | $1.08 \times 10^{-7}$ | $\text{mL.pg}^{-1}.\text{min}^{-1}$ |
| $\delta_F$ | $2.35 \times 10^{-12}$ | $\text{min}^{-1}$ |
| $\lambda_{F1}$ | 1.063 | $\text{ng}^{-1}$ |
| $\lambda_{F2}$ | 64.51 | $\text{g}^{-1}$ |
| $\epsilon$ | $2.093 \times 10^{-8}$ | $\text{g}^{-1}$ |
| $\tau$ | 2 | day |
| $\tau'$ | 10 | day |
| $\Delta_0$ | $2.614 \times 10^{-5}$ | $\text{min}^{-1}$ |

**Table IV.1** – Parameter values estimated for System (IV.4), on groups H1 and AL

#### IV.3.3.1 Experimental data from [Jacquier et al., 2014]

The model is first tested on experimental data from [Jacquier et al., 2014]. These data correspond to a temporal record for 8 weeks of food intake and body weight, with initial and final values for ghrelin, leptin and glucose. The data were obtained on 5 groups of 6 rats (see Section II.2.1 for more details):

- group D0: sacrificed on day 0 to obtain initial condition,
- group AL: 8 weeks of *Ad libitum* feeding,
- group H0: 8 weeks of caloric restriction, with a constant amount of food available each day,
- group H1: 8 weeks of caloric restriction, with daily food availability changing each week,
- group H4: 8 weeks of caloric restriction, with daily food availability changing every 4 weeks (alternation of low and high food availability).

As the equation describing body weight dynamics is modified, the estimation of parameter values is performed similarly to the one presented in Section II.2.4 and concerns parameters related to body weight, food intake and energy expenditure: $\gamma_M$, $\gamma_F$, $\delta_F$, $\lambda_{F1}$, $\lambda_{F2}$, $\epsilon$, $\tau$ and $\tau'$ (see Table IV.1), as well as the initial condition for the rate of energy expenditure $\Delta_0$. Other parameter values are taken from Table II.4. With these parameters values, $A_2 < 0$ yet the system displays numerically a single positive equilibrium.

The prediction of body weight and food intake for all groups of rats is similar to what was obtained with the model taking into account the dynamics of fat mass and fat-free mass (see Table IV.2), and reproduces experimental data (see Figures IV.4, IV.5 and IV.6).

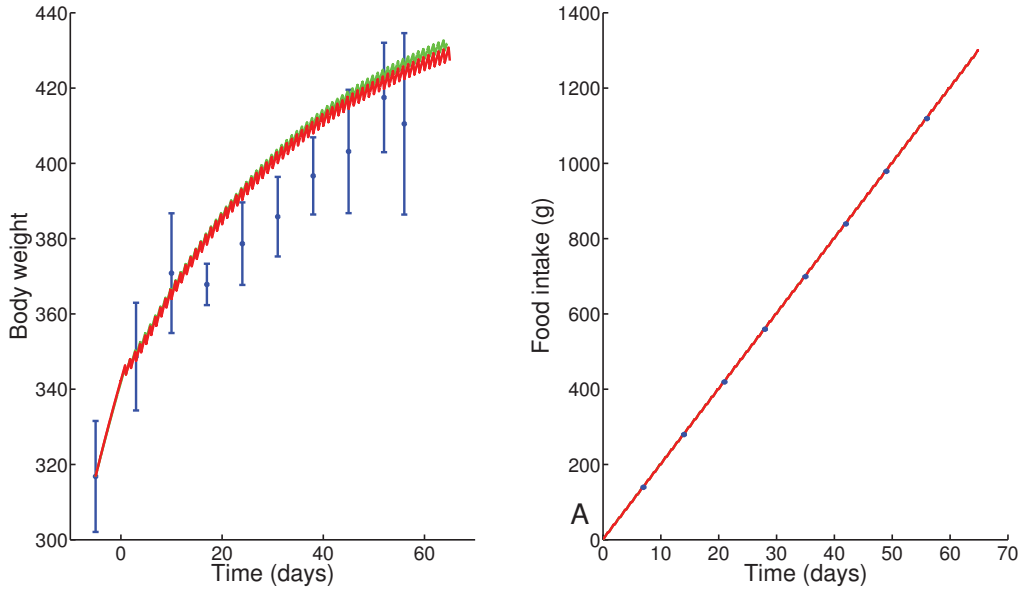


**Figure IV.4** – Predictions of System (IV.4) (in green) and System (II.1)–(II.8) (in red) for body weight and food intake compared to experimental data (mean±sd, in blue) for group H0, with parameter values from Table IV.1.

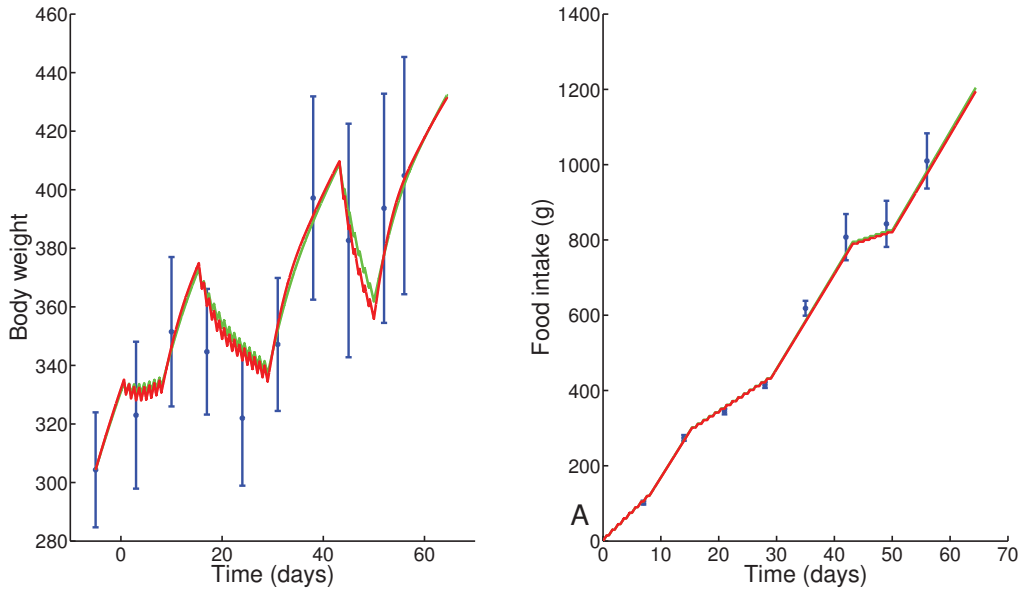


**Figure IV.5** – Predictions of System (IV.4) (in green) and System (II.1)–(II.8) (in red) for body weight and food intake compared to experimental data (mean±sd, in blue) for group H1, with parameter values from Table IV.1. This group of rats was used to estimate parameter values relative to the adaptation of energy expenditure.

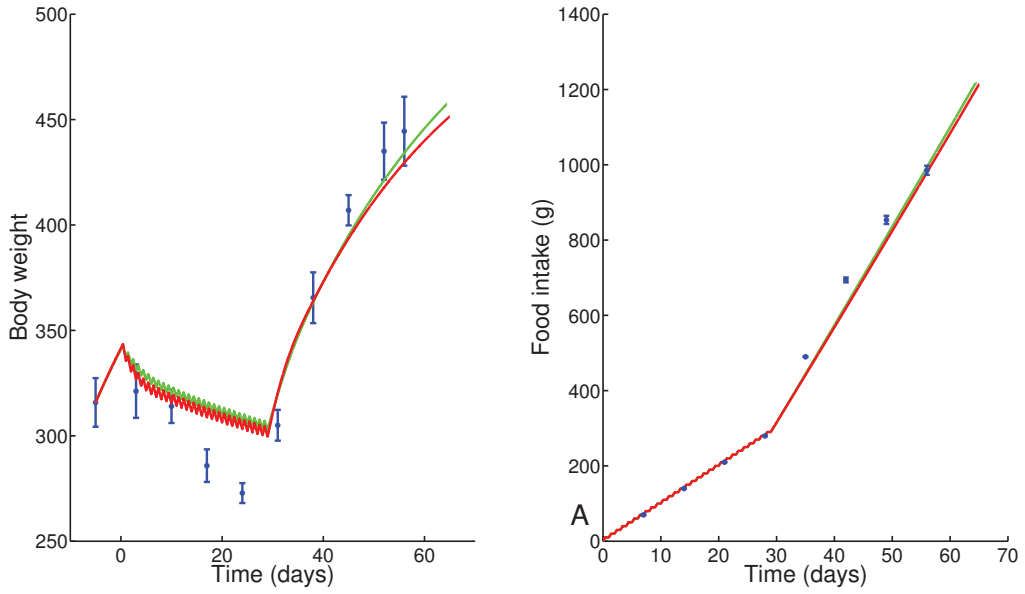


**Figure IV.6** – Predictions of System (IV.4) (in green) and System (II.1)–(II.8) (in red) for body weight and food intake compared to experimental data (mean±sd, in blue) for group H4, with parameter values from Table IV.1.

| Model | Group | AIC |
|---|---|---|
| System (II.1)–(II.8) | H0 | 405.3 |
| | H1 | 411.5 |
| | H4 | 466 |
| System (IV.4) | H0 | 405.9 |
| | H1 | 465.4 |
| | H4 | 411.8 |

**Table IV.2** – Comparison of the Akaike information criteria (AIC) obtained with System (II.1)–(II.8) and System (IV.4) for the data from [Jacquier et al., 2014].

### IV.3.3.2 Long-term dynamics

In addition to data from [Jacquier et al., 2014], the behavior of the model is tested on the long-term, for 16 weeks. As in the previous section, the data set used corresponds to caloric restriction experiments on 2 groups of Wistar rats. Both groups of rats received the same total amount of food over 16 weeks, with different patterns of food availability: one group received a constant amount of food each day (denoted as $H0_{16w}$) and the other one received an alternation between a high amount of food for approximately 4 weeks and low food availability for 4 weeks (denoted as $H4_{16w}$). During the experiments, some biopsies were performed to determine the characteristics of the adipose tissue. At the

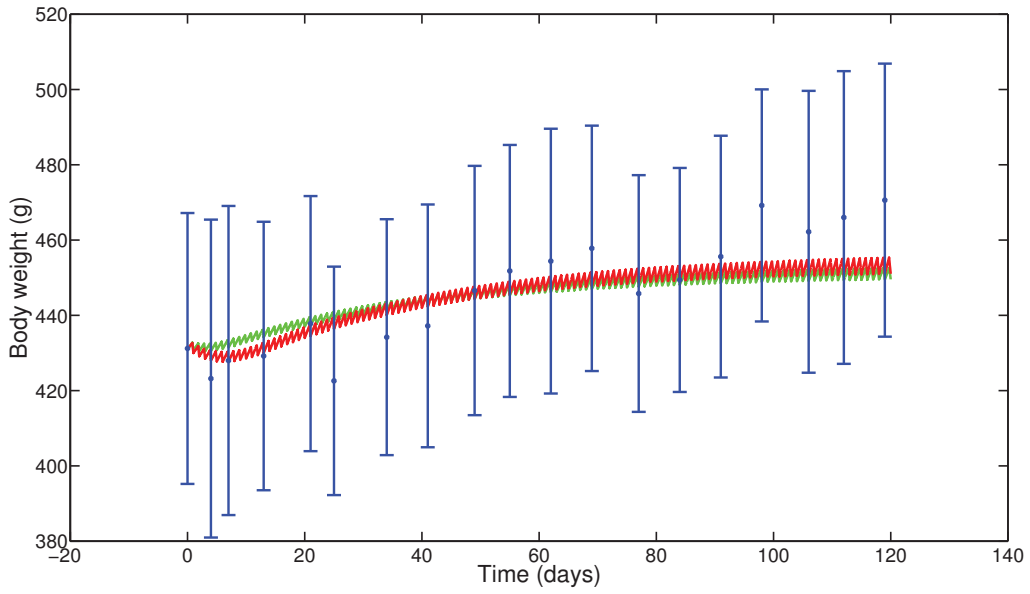


**Figure IV.7** – Body weight evolution predicted by System (IV.4) (in green) and System (II.1)–(II.8) (in red) compared to experimental body weight (error bars, in blue) for 16 weeks on the group with constant food availability ($H0_{16w}$). For System (II.1)–(II.8), original parameter values from Chapter II were used.

| Model | Group | $RSS/p$ |
|---|---|---|
| System (II.1)–(II.8) | $H0_{16w}$ | 1043.3 |
| | $H4_{16w}$ | 979.8 |
| System (IV.4) | $H0_{16w}$ | 1063.55 |
| | $H4_{16w}$ | 909.4 |

**Table IV.3** – Comparison of the Residual Sum of Squares $RSS$ (divided by the number of data points $p$) obtained with System (II.1)–(II.8) and System (IV.4) for the data from [Jacquier et al., 2014].

end of the 16 weeks, despite a significant difference in food consumption, rats from both groups had similar biometric data (no significant difference, data not shown).

First, the predictions of the original model (System (II.1)–(II.8)) are compared to this data set. To do this, it is possible to use parameter values estimated in [Jacquier et al., 2014] or to estimate new parameter values. Then, System (IV.4) is used and compared to experimental data and to the results from the System (II.1)–(II.8) (see Table IV.3). In both cases, the only input of the system is the food availability (corresponding to function $f(t)$ in System (IV.4)). System (IV.4), using the parameter values determined in Section IV.3.3.1, gives good predictions of the evolution of body weight for 16 weeks without the need to estimate new parameter values for both groups of rats (see Figures IV.7 and IV.8,

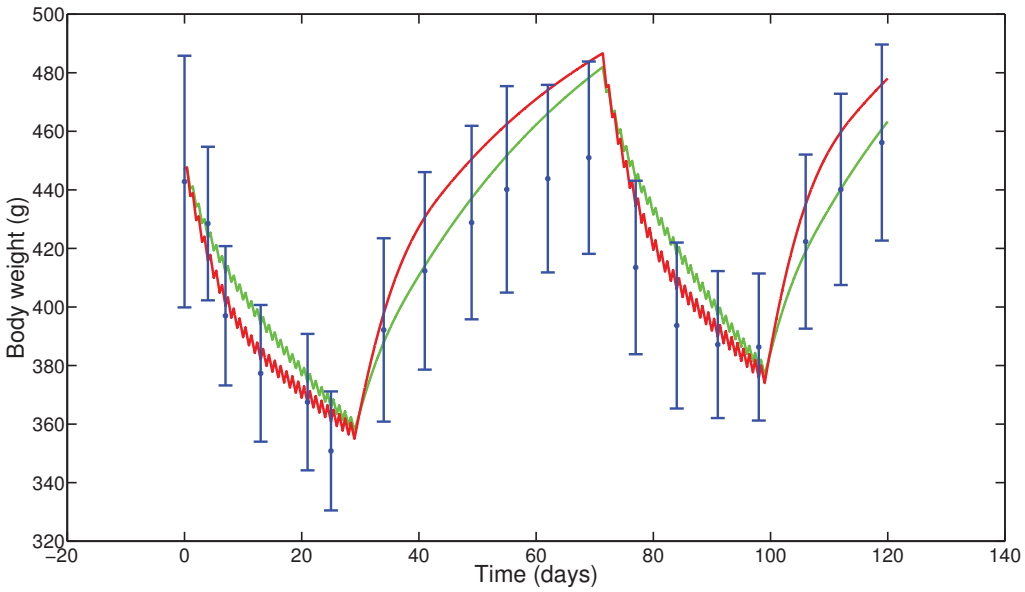


**Figure IV.8** – Body weight evolution predicted by System (IV.4) (in green) and System (II.1)–(II.8) (in red) compared to experimental body weight (error bars, in blue) for 16 weeks on the group with alternations between low and high food availability ($H4_{16w}$). For System (II.1)–(II.8), original parameter values from Chapter II were used.

green curves, and Table IV.3). The results of the model defined by System (IV.4) are similar to the results of System (II.1)–(II.8) without estimating new parameter values (see Figures IV.7 and IV.8, green and red curves).

## IV.4 Leptin resistance

In this section, we study the new version of the model of the dynamics of food intake and body weight with leptin regulation of food intake via leptin receptors presented in Chapter III. Body weight and leptin dynamics will now be described by Equations (IV.1) and (IV.2), instead of Equations (III.1), (III.2) and (III.6). The new system will be analyzed and simulated to be compared to experimental data and to the results obtained in Chapter III, in order to validate the simplification presented in Section IV.2.

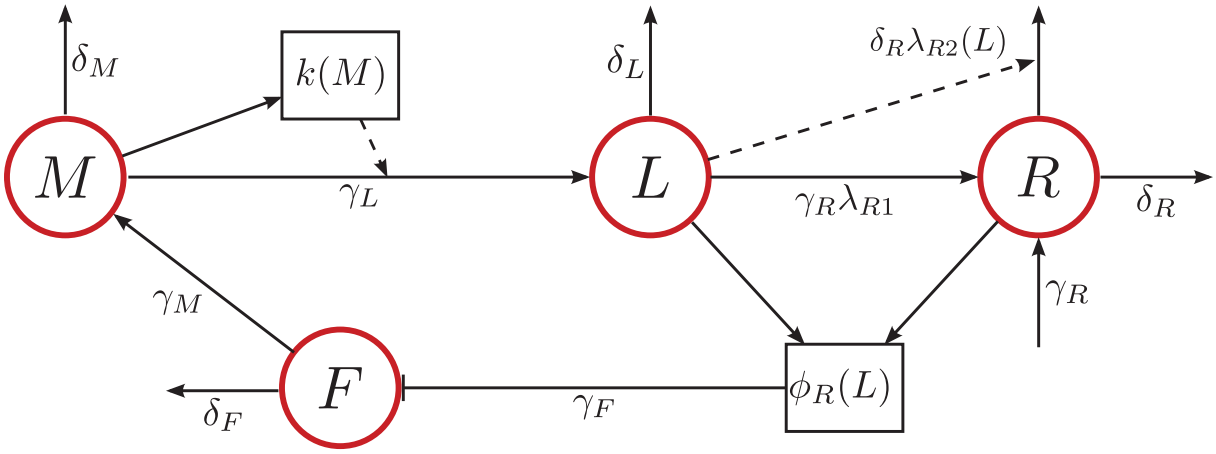


**Figure IV.9** – Variable flow diagram for the model described by System (IV.7), accounting for leptin resistance. Bar-headed lines indicate an inhibition, straight arrows a production and dashed arrows represent an influence on the degradation or the production. Lines ending with a dot indicate positive or negative influence (depending on the variable and parameter values).

### IV.4.1 Model

We denote body weight by $M$, food intake by $F$, leptin by $L$ and leptin receptors by $R$ (see Figure IV.9). The resulting system for leptin resistance, based on System (III.10), is defined as follows:

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \gamma_M F - \delta_M M, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L k(M) M - \delta_L L, \\ \dfrac{\mathrm{d}R}{\mathrm{d}t} &= \gamma_R f_1(L) - \delta_R f_2(L) R, \\ \dfrac{\mathrm{d}F}{\mathrm{d}t} &= \dfrac{\gamma_F}{1+\Phi_R(L)} - \delta_F F, \end{cases} \tag{IV.7}$$

with $f_1$ and $f_2$ two increasing functions of $L$, verifying that for $L$ large $f_2(L) \gg f_1(L)$ and $\Phi_R(L)$ a function describing the result of the activation of leptin receptors by leptin, as described in Chapter III.

### IV.4.2 Analysis

In order to simplify the analysis of System (IV.7) while keeping most of its properties, we consider the following assumptions:

1. the activation of leptin receptors by leptin is proportional to the number of receptors and does not depend on leptin concentration: $\Phi_R(L) = \Psi R$,

2. $f_1(L) = 1$ and $f_2(L) = L$, which are the simplest functions verifying the condition $f_2(L) \gg f_1(L)$ for $L$ large.

System (IV.7) thus becomes:

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \gamma_M F - \delta_M M, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L k(M) M - \delta_L L, \\ \dfrac{\mathrm{d}R}{\mathrm{d}t} &= \gamma_R - \delta_R L R, \\ \dfrac{\mathrm{d}F}{\mathrm{d}t} &= \dfrac{\gamma_F}{1 + R\Psi} - \delta_F F, \end{cases} \tag{IV.8}$$

An equilibrium point $(M^*, L^*, R^*, F^*)$ of System (IV.8) satisfies the conditions:

$$L^* = \frac{\gamma_L}{\delta_L} k(M^*) M^*, \tag{IV.9}$$

$$R^* = \frac{\gamma_R}{\delta_R L^*}, \tag{IV.10}$$

$$F^* = \frac{\gamma_F}{\delta_F (1 + \Psi R^*)}, \tag{IV.11}$$

$$F^* = \frac{\delta_M}{\gamma_M} M^*. \tag{IV.12}$$

From (IV.11) and (IV.12), and using Equations (IV.9) and (IV.10), we can define the functions $F_1(M)$ and $F_2(M)$, as follows:

$$F_1(M) = \frac{\delta_M}{\gamma_M} M \tag{IV.13}$$

$$F_2(M) = \frac{\gamma_F \gamma_L \delta_R k(M) M}{\delta_F (\gamma_L \delta_R k(M) M + \gamma_R \delta_L \Psi)} \tag{IV.14}$$

An intersection between $F_1$ and $F_2$ defines a value for $F^*$ and thus an equilibrium of System (IV.8).

To study the existence and stability of equilibria, we determine the Jacobian matrix $J$ of System (IV.8) at a given equilibrium $(M^*, L^*, R^*, F^*)$, given by

$$J = \begin{pmatrix} -\delta_M & 0 & 0 & \gamma_M \\ \gamma_L \frac{\partial(k(M)M)}{\partial M} & -\delta_L & 0 & 0 \\ 0 & -\delta_R R^* & -\delta_R L^* & 0 \\ 0 & 0 & \frac{-\gamma_F \Psi}{(1+R^*\Psi)^2} & -\delta_F \end{pmatrix},$$

As we defined $k(M)$ as an increasing function of $M$, $\frac{\partial(k(M)M)}{\partial M}$ is positive.

The characteristic polynomial at the equilibrium point is then defined as:

$$P(\chi) = \chi^4 + A\chi^3 + B\chi^2 + C\chi + D, \tag{IV.15}$$

with

- $A = \delta_M + \delta_L + \delta_F + \delta_R L > 0$
- $B = \delta_M\delta_L + \delta_F\delta_R L + (\delta_M + \delta_L)(\delta_F + \delta_R L) > 0$
- $C = \delta_M\delta_L(\delta_F + \delta_R L) + \delta_F\delta_R L(\delta_M + \delta_L) > 0$
- $D = \delta_M\delta_L\delta_F\delta_R L - \frac{\partial(k(M)M)}{\partial M}\frac{\gamma_M\gamma_L\gamma_F\Psi\delta_R R}{(1+\Psi R)^2}$

According to Routh-Hurwitz criteria, all roots of the characteristic polynomial $P(\chi)$ are negative or have negative real parts and thus the equilibrium $(M^*, L^*, R^*, F^*)$ is stable if all the following conditions are satisfieded:

- $D > 0$,
- $AB > C$, which is always satisfied,
- $ABC > C^2 + A^2D$, which is always satisfied.

Then, if $D > 0$, $P$ has no positive real root nor complex root with a positive real part, and thus the equilibrium point is stable. $D > 0$ is equivalent to $F_1'(M) > F_2'(M)$ at the equilibrium point, assuming that $k(M)M \neq 0$. An equilibrium point$(M^*, L^*, R^*, F^*)$ of System (IV.8) is then stable if and only if $F_1'(M^*) > F_2'(M^*)$. This is the same condition than the one obtained in Chapter III (Equation (A.8)).

We can numerically compute the equilibrium points and their stability. Depending on the parameter values, System (IV.8) has 1 stable positive equilibrium or 3 positive equilibria (two stable, one unstable). Similarly to Chapter III, we can define 2 stable equilibria with low body weight (healthy state) or high body weight (obese and leptin resistant state). When varying a certain parameter value, one obtains a bifurcation diagram with a hysteresis (see Figures IV.10 and IV.11).

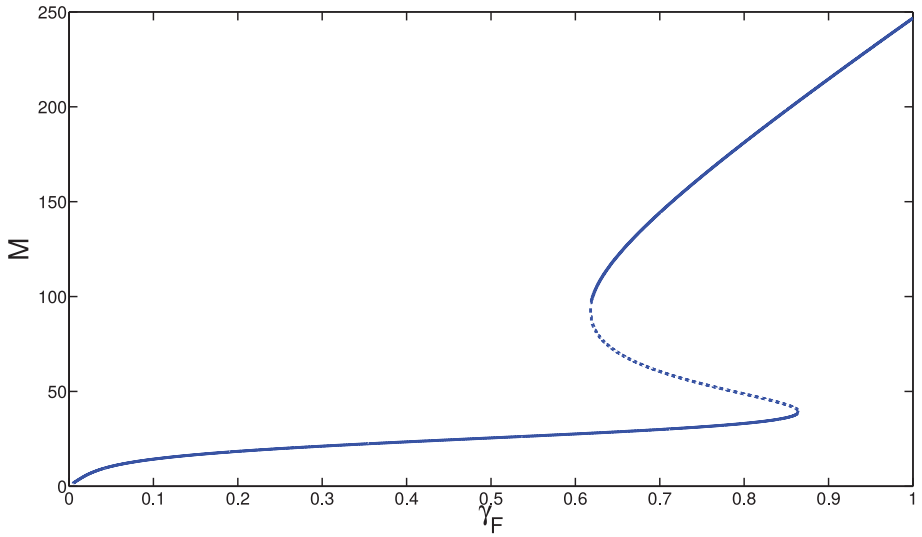


**Figure IV.10** – Bifurcation diagram for the new leptin resistance model defined by System (IV.8), with $k(M) = aM^2/(M^2 + b)$, for parameter $\gamma_F$ varying from 0 to 1. The system displays between one and three equilibria, stable equilibria correspond to a healthy state or a leptin resistant and obese state.

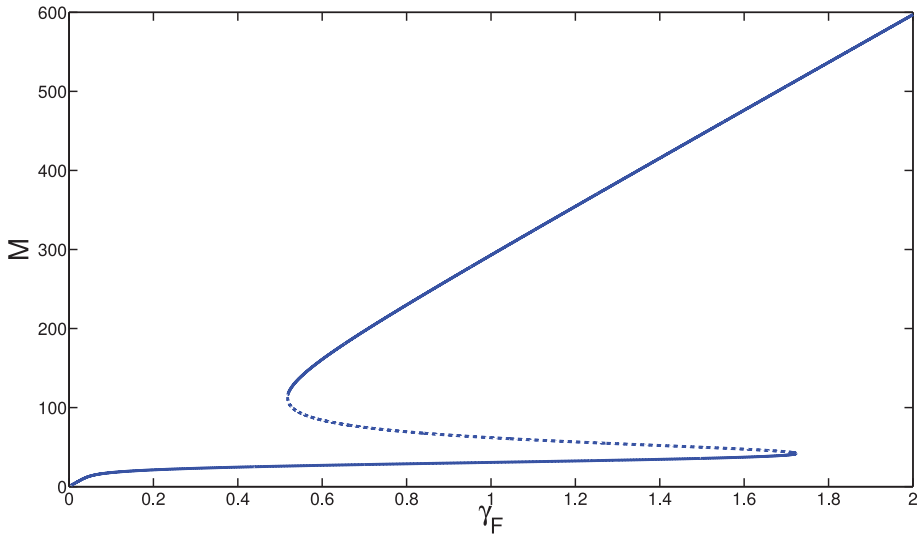


**Figure IV.11** – Bifurcation diagram for the new leptin resistance model (System (IV.7), with $k(M) = aM^2/(M^2 + b)$, $f_1(L) = 1 + \lambda_{R1}L$ and $f_2(L) = 1 + \lambda_{R2}L^2$) for parameter $\gamma_F$ varying from 0 to 2. The system displays between one and three equilibria, stable equilibria correspond to a healthy state or a leptin resistant and obese state.

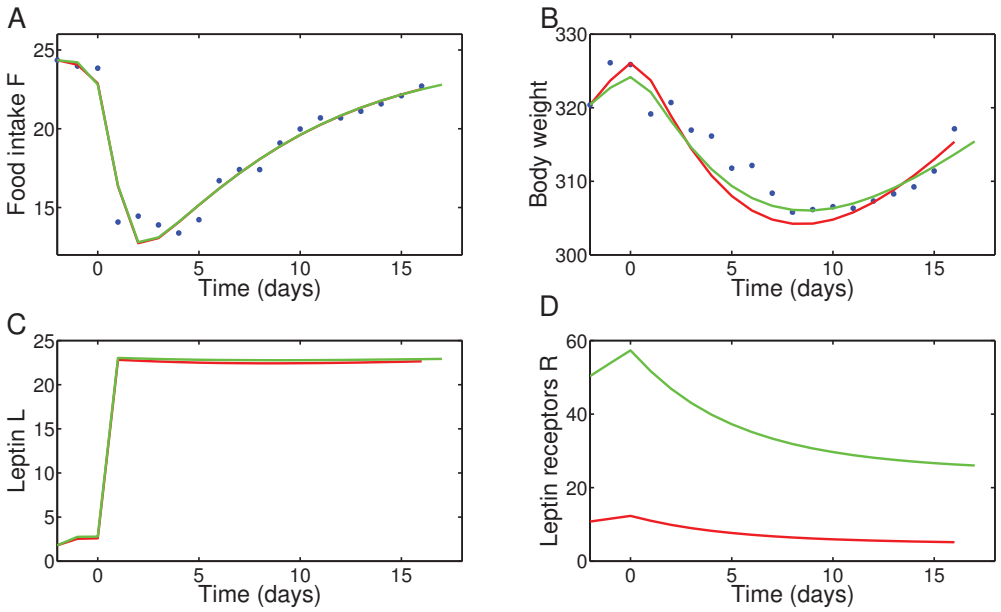


**Figure IV.12** – Predictions obtained with the original ( System (III.10), red lines) and the new model (System (IV.7), green line) with a constant leptin injection starting at day 0, compared to experimental data (blue dots) [Pal and Sahu, 2003]. Parameter values used for these simulations have been obtained by minimizing the residual sum of squares for food intake (see Table IV.4). System (IV.7) gives results similar to System (III.10) despite having less parameters. A. Food intake $F$ prediction is similar for both models, with an important decrease from day 0 followed by a return to the initial value from day 4. B. Body weight $M = FM + FFM$ starts to decrease at the beginning of the injection until day 8 and then progressively increases. C.Leptin concentration $L$, initially low, reaches a very high value after the injection, which is predicted by both models. D. Leptin receptors $R$ predictions do not correspond to the same range of variations for both model but the evolution is the same: an increase before the injection followed by an important decrease starting at day 0.

### IV.4.3 Comparison to experimental data

In order to test the validity of this model, we compare its ability to reproduce experimental data from [Pal and Sahu, 2003] to the result obtained in Chapter III (see Figures IV.12 and IV.13). Experimental data are obtained by monitoring food intake and body weight during a 16 days leptin infusion in the central nervous system of rats. We used System (IV.7) with:

$$\begin{aligned} k(M) &= \frac{aM^2}{M^2+b}, & \Phi(L) &= \frac{\phi L^n}{L^n+\theta^n}, \\ f_1(L) &= 1+\lambda_{R1}L, & f_2(L) &= 1+\lambda_{R2}L^2. \end{aligned}$$

Parameter values are estimated from experimental data (see Table IV.4), except for $k(M)$ (see Section IV.2.2), by minimizing the residual sum of squares between the observations and the prediction. We can consider two sets of experimental data for this minimization,

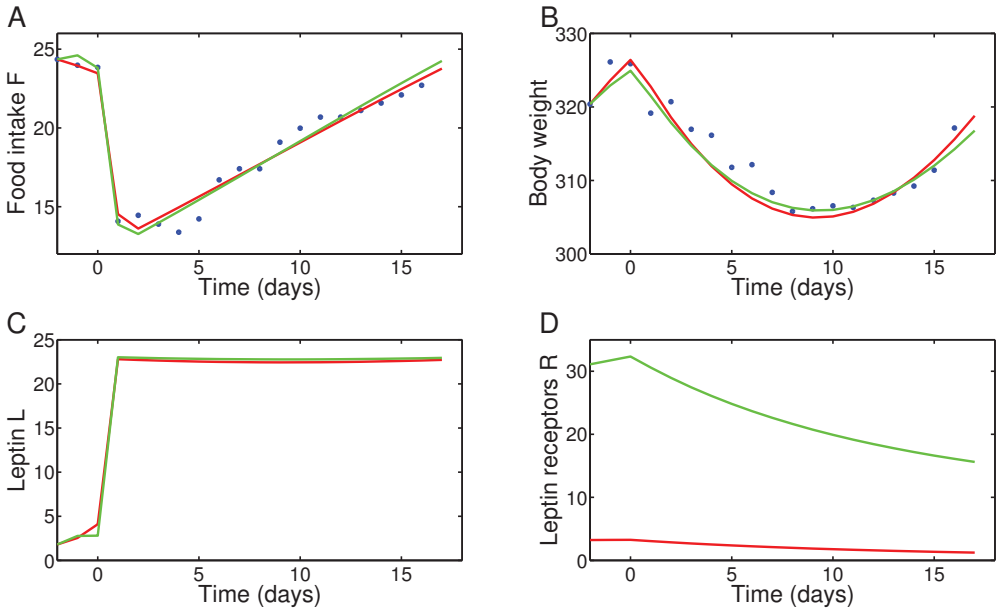


**Figure IV.13** – Predictions obtained with System (III.10) (red lines) and System (IV.7) (green line) with a constant leptin injection starting at day 0, compared to experimental data (blue dots) [Pal and Sahu, 2003]. Parameter values used for these simulations have been obtained by minimizing the residual sum of squares for food intake and body weight (see Table IV.4). Compared to Figure IV.12, the behavior of all variables is similar but the prediction is more accurate for body weight and less accurate for food intake.

| Parameter | Value (estimated for $F$) | Value (estimated for $F$ and $M$) | Unit |
|---|---|---|---|
| $\gamma_M$ | $0.548$ | | $\text{min}^{-1}$ |
| $\delta_M$ | $2.37 \times 10^{-5}$ | | $\text{min}^{-1}$ |
| $a$ | $0.232$ | | N.U. |
| $b$ | $77523$ | | $\text{g}^2$ |
| $\gamma_R$ | $3.18 \times 10^{-3}$ | $4.86 \times 10^{-4}$ | $\text{mol.L}^{-1}.\text{min}^{-1}$ |
| $\delta_R$ | $1.24 \times 10^{-5}$ | $1.22 \times 10^{-6}$ | $\text{min}^{-1}$ |
| $\lambda_{R1}$ | $4.23 \times 10^{-5}$ | $3.6 \times 10^{-4}$ | $\text{ng}^{-1}$ |
| $\lambda_{R2}$ | $4.59 \times 10^{-5}$ | $2.17 \times 10^{-4}$ | $\text{ng}^{-2}$ |
| $\gamma_F$ | $5.039 \times 10^{-4}$ | $1.69 \times 10^{-3}$ | $\text{g.min}^{-1}$ |
| $\delta_F$ | $1.18 \times 10^{-3}$ | $6.01 \times 10^{-3}$ | $\text{min}^{-1}$ |
| $\theta$ | $57.11$ | $55.59$ | ng |

**Table IV.4** – Estimated parameter values for the new model with leptin resistance (System (IV.7)). These values have been obtained by minimizing the residual sum of squares for food intake or food intake and body weight, as indicated. Parameters $\gamma_M$ and $\delta_M$ have been obtained separately by estimating the evolution of body weight as a function of food intake. Parameters $a$ and $b$ have been obtained as described in Section IV.2.2. Parameters not displayed in this table are the same as in Table III.3.

| Variables used | Model | $RSS$ | Parameters $p$ | Data points $n$ | $AIC$ |
|---|---|---|---|---|---|
| $F$ | System (III.10) | 5.25 | 16 | 19 | 7.56 |
| | System (IV.7) | 5.11 | 12 | 19 | -0.95 |
| $F$ and $M$ | System (III.10) | 114.1 | 16 | 38 | 73.78 |
| | System (IV.7) | 91.39 | 12 | 38 | 57.34 |

**Table IV.5** – Residual sum of squares ($RSS$) and Akaike information criterion ($AIC$) for the original model (from Chapter III) and the new model, with experimental data on leptin injection from [Pal and Sahu, 2003]. The RSS is computed for the variables $F$ and $M$. The Akaike information criterion is calculated as follows : $AIC = n\ln(RSS/n) + 2p$, with $p$ the number of parameters and $n$ the number of data points.

the first one is composed only by food intake so we fit the experimental food intake to the observed one (see Figure IV.12), the second one is composed of both food intake and body weight to improve body weight prediction (see Figure IV.13). Both model predictions are close to experimental data for food intake and body weight. The only difference concerns the density of leptin receptors $R$, which is higher for the model presented in this chapter compared to the model from Chapter III. However, the variations predicted for leptin receptors are the same for both models: an initial increase followed by a decrease to a value lower than the initial condition. The behavior of the system is the same if the parameter values are estimated by fitting either food intake only or by fitting both food intake and body weight, despite slightly different parameter values (see Table IV.4). As these parameter values are not available in the literature, it is difficult to determine if the sets of parameter values obtained are realistic. However, the predictions are coherent with experimental data. With these parameter values, the system is monostable with a healthy state but with the addition of a constant leptin infusion, the equilibrium changes and corresponds to the obese and leptin resistant state.

The residual sum of squares, minimized to estimate parameter values, is similar for both the new model (System (IV.7)) and System (III.10) (see Table IV.5) when the minimization is performed on food intake or food intake and body weight. We can then compute the Akaike Information Criterion to determine the more adapted model for this data set (see Table IV.5). Thus, the $AIC$ is lower for the new system, which has less parameters, so we can conclude that this model is more adapted to reproduce the experimental data on leptin infusion from [Pal and Sahu, 2003].

We can also test the effect of parameter variations on the dynamics of the system, similarly to what was done in Chapter III when varying parameter $\gamma_F$. Results are similar to the complete model (see Figures III.7 and III.9), with a possibility to switch to another

equilibrium when parameter values change (not shown).

Regarding the modeling of food intake and body weight regulation with leptin resistance, System (IV.7) gives similar or better results than System (III.10). In this case the simplification of body weight dynamics is a success, with a system without positivity issues and generating better results. As the new model gives good results for the models considering hormonal regulation and leptin dynamics, it would then be interesting to combine all equations in order to have a more complete description of body weight dynamics with hormonal regulation.

## IV.5 A model of body weight and food intake dynamics including hormonal regulation and leptin receptors dynamics

As we have seen earlier in this manuscript, hormonal regulation of food intake and energy expenditure is an important component of body weight regulation. It can then be interesting to combine both the hormonal regulation of food intake and the development of leptin resistance in the same model, allowing to complexify the mechanisms of regulation and to take into account multiple scenarii. In this section, we present a model considering the regulation of food intake, body weight and energy expenditure by hormones leptin and ghrelin, glucose and leptin receptors.

The model considered in this section (see Figure IV.14) is a combination of Systems (IV.4) and (IV.7). It provides a complete description of the regulation of food intake and body weight by hormones, and allows the description of leptin resistance. The dynamics of body weight $M$, plasma glucose $U$, plasma ghrelin $E$, plasma leptin $L$, leptin receptors $R$, expected food intake $F$, rate of energy expenditure $\Delta$ and available food $A$ are considered,

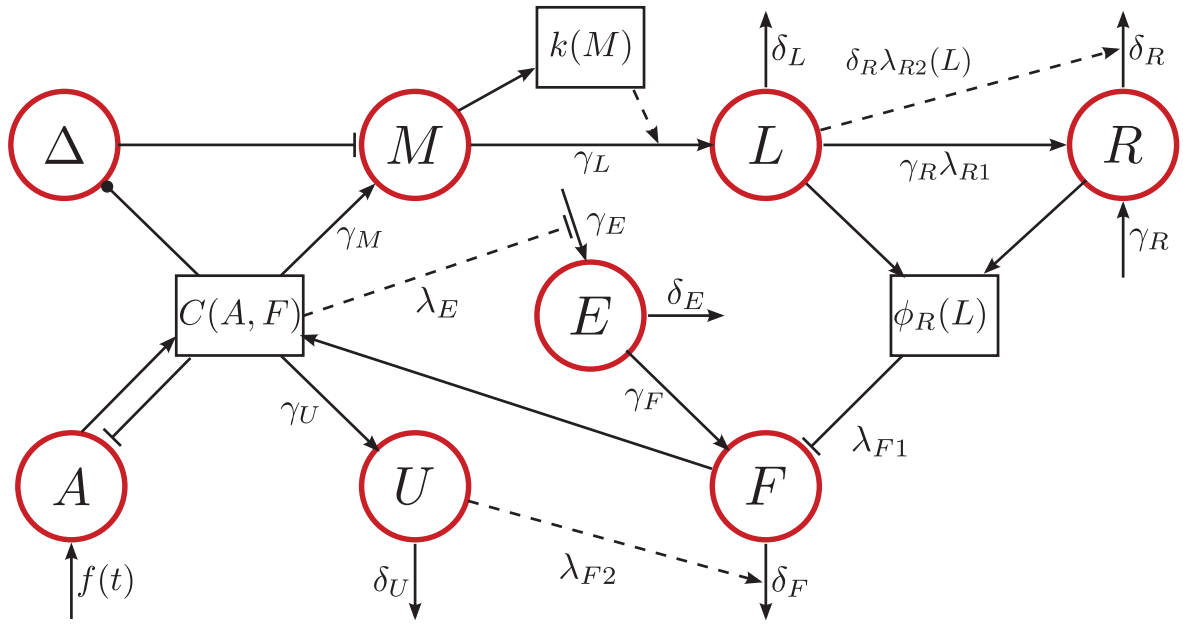


**Figure IV.14** – Variable flow diagram of System (IV.16), describing the dynamics of body weight and food intake, taking into account leptin, leptin receptors, ghrelin, glucose, adaptation of the rate of energy expenditure $\Delta$ and food availability. Bar-headed lines indicate an inhibition, straight arrows a production and dashed lines represent a positive or negative influence on the degradation or the production. Lines ending with a dot indicate positive or negative influence (depending on the variable and parameter values).

as follows

$$\begin{cases} \dfrac{\mathrm{d}M}{\mathrm{d}t} &= \gamma_M C(A,F) - \Delta M, \\ \dfrac{\mathrm{d}U}{\mathrm{d}t} &= \gamma_U C(A,F) - \delta_U U, \\ \dfrac{\mathrm{d}E}{\mathrm{d}t} &= \dfrac{\gamma_E}{1+\lambda_E C(A,F)} - \delta_E E, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L k(M) M - \delta_L L, \\ \dfrac{\mathrm{d}R}{\mathrm{d}t} &= \gamma_R(1+\lambda_{R1}L) - \delta_R(1+\lambda_{R2}L^2)R, \\ \dfrac{\mathrm{d}F}{\mathrm{d}t} &= \dfrac{\gamma_F E}{1+R\Phi(L)} - \delta_F(1+\lambda_{F2}U)F, \\ \dfrac{\mathrm{d}\Delta}{\mathrm{d}t} &= \epsilon\left(\dfrac{1}{\tau}\displaystyle\int_{t-\tau}^{t} C(A(v),F(v))\mathrm{d}v - \dfrac{1}{\tau'}\int_{t-\tau'}^{t} C(A(v),F(v))\mathrm{d}v\right), \\ \dfrac{\mathrm{d}A}{\mathrm{d}t} &= f(t) - C(A,F), \end{cases} \tag{IV.16}$$

with $\Phi(L) = \phi L^n/(L^n + \theta^n)$. Due to the description of leptin receptors, the equation describing the expected food intake $F$ has to be modified compared to System (IV.4) to include the activation of leptin receptors by leptin leading to leptin action on food intake.

Due to its construction, System (IV.16) should have the same properties as the previous models, in particular the existence of a healthy state and a leptin resistant state, depending on parameter values.

This model can be used, for instance, to study the impact of changes in food availability on the development of leptin resistance. This can be achieved by modifying the function $f(t)$ and potentially the composition of the food (parameters $\gamma_M$, $\gamma_U$ and $\lambda_E$). This model represents a unique and novel description of body weight regulation by hormones and includes mechanisms describing the development of leptin resistance. Its detailed study and application to biological questions are however left for future works.

# Chapter V

# Discussion and perspectives

When applied to the dynamics of food intake and body weight, mathematical and computational modeling is an efficient and powerful tool to study the hormonal regulation of such complex biological processes. Built on multiple assumptions based on biological knowledge, the models are used complementarily to experiments to analyze and predict the behavior of these systems.

In Chapter II, I presented a model of the dynamics of body weight, food intake and energy expenditure in rats, regulated by hormones (leptin and ghrelin) and glucose concentration. This model includes the availability of food and a memory of the previous food intake, estimated around 8 days, which induces variations in energy expenditure to reduce energy balance, and thus limits changes in body weight. Dedicated experiments of caloric restriction were conducted by submitting three groups of rats to different patterns of food availability with the same global amount of available food, along with a control group with *Ad libitum* food. These experiments show that the different patterns of food intake induce differences in body weight dynamics that cannot be explained only by the food consumption. The model is applied on this specific data set and is able to predict the evolution of food intake and body weight in all groups of rats, due to the low variability between these animals, showing the importance of the memory of the food intake in the regulation of energy expenditure and body weight. When applied to a new set of similar experimental data the model gives correct predictions, without changes in parameter values, highlighting its predictive aspect on this strain of rats (Wistar rats).

The delay in the adaptation of energy expenditure allows to limit the loss of body weight when food is reduced but also induces an important gain of body weight when the starva-

tion ends, because the energy expenditure was adapted to the reduced food consumption. Important variations in food intake tend to induce weight gain, in particular an important reduction of food intake induces a decrease in energy expenditure, leading to gains of body weight and fat mass when the food intake becomes higher. In the model, this phenomenon is reversible, as energy expenditure can also increase, which may not be the case in reality. The patterns of food availability can then have a more important impact on the evolution of body weight than the total food consumption.

In Chapter III, I presented a model describing the dynamics of food intake and body weight, regulated by leptin and leptin receptors, to account for the development of leptin resistance. The dynamics of body weight in this model are based on Chapter II's model, without considering regulators other than leptin nor the adaptation of the rate of energy expenditure. The downregulation of food intake by leptin is performed through the activation of leptin receptors, and then depends on the amount of receptors and the concentration of leptin. Leptin is assumed to be a regulator of its own receptors, depending on its concentration: if leptin concentration is important, leptin receptors are downregulated. This system can have up to two stable positive equilibria depending on parameter values, corresponding to a healthy state (with low fat mass and the absence of leptin resistance) and a leptin-resistant/obese state (high fat mass, high leptin concentration, low number of receptors). I showed that, starting from the healthy state, a constant leptin infusion can induce leptin resistance, due to an important decrease in leptin receptors. This was tested against experimental data from [Pal and Sahu, 2003], and the model is able to reproduce the evolution of body weight and food intake observed experimentally, as well as the development of leptin resistance.

Modifications in leptin concentration are not the only potential cause of leptin resistance and temporal modifications of parameter values can induce a change of equilibrium and then the development of leptin resistance and obesity. In particular, I showed that a progressive increase in food intake stimulation can induce a pathway from the healthy state to the leptin-resistant/obese state. However, the inverse modification in the parameter value does not always induce a return to the initial state, due to a hysteresis cycle. A recurrent increase in food consumption can then, after some time, induce a state of leptin resistance and obesity, without being easily reversible. Other possible modifications of food intake stimulation include oscillations, which result in oscillations of body weight around the healthy state, the obese state or between the two states, depending on the frequency and amplitude of the variation. The probability to develop leptin resistance and obesity when submitted to changes in parameter values is dependent on the initial

value for this parameter and the initial state (healthy or leptin-resistant/obese). The description of leptin receptors in this model has an important impact on the dynamics of food intake and body weight and their regulation by leptin is then an important element in the development of leptin resistance.

In Chapter IV, I presented a realistic simplification of the equations describing the dynamics of fat and fat-free mass, which results in a single ordinary differential equation describing body weight. Fat mass is then described as an increasing function of body weight, which is fitted to experimental data. The new equation and the description of fat mass are included in the models described in Chapters II and III instead of the description of fat mass and fat-free mass previously used. With this new equation, the analysis of the models is simplified and there is no need to limit the range of parameters values to maintain the positivity of the solutions. The new and simplified version of the models can then be compared to the original models on their ability to reproduce experimental data: the results are as good as the previous models' or even better in the case of the development of leptin resistance during leptin infusion, with less parameter values to estimate. We can then build a new model, combining both previous models to build a complete description of the regulation of body weight and food intake by hormones, which can account for leptin resistance, adaptation of energy expenditure and caloric restrictions.

The last model presented in Chapter IV presents a complete picture of regulation of food intake and body weight by hormones, with the possibility to describe leptin resistance and potentially the development of obesity. However, it has not been deeply analyzed nor compared to experimental data so it would be interesting to continue the study of this model. It would be particularly relevant to find or generate data on the development of leptin resistance with different conditions to test the predictions of the model. For instance, one may think that the important oscillations of body weight induced by an alternation between low and high food intake could induce leptin resistance, as there is an important fat accumulation during the periods of high food intake. Another potential pathway to obesity is aging, which impacts the efficiency of the regulations or could even cause the disruption of some pathways.

All the comparisons of the models with experimental data were performed by fitting the behavior of the entire population and not of individuals. This is possible in rats, due to the genetic and physiological homogeneity of the population considered. When considering populations other than laboratory rodents, the variability between individuals can be important, and increases with time. A way to consider individuals is to use mixed-effects modeling, which accounts for fixed (population) and random (individuals) parts in the

parameter values.

As we have not included every regulator of food intake and body weight in the models, most of the possible extensions of these models include a complexification of the dynamics, thus limiting the possibility of analysis. Maybe the first extension to this work would be to reduce the systems to the equations with the most impact on the dynamics. For example, we showed in the model describing the development of leptin resistance that a system with only leptin as a regulator of food intake was able to predict experimental data. Then we could compare the behavior of a model without the regulations by ghrelin and glucose to the model with these regulations, to determine if they are really useful to explain the dynamics. Some equations are more important for the dynamics, such as the dynamics of the rate of energy expenditure, which we showed to be necessary to explain experimental data during caloric restriction, but not when the food availability is *Ad libitum*.

In Chapter II, hormones leptin and ghrelin, and glucose were considered as regulators of food intake. Insulin was not included in the model, as it is highly correlated with glucose. However, as we have seen in Chapter I, insulin can be considered as an adiposity feedback signal as well as leptin, and insulin interacts with leptin production, and to a lesser extent with ghrelin production. Thus, it could be interesting to include explicitly the dynamics of insulin in the model, its interactions with glucose and other hormones. Mathematical models describing the regulation of plasma glucose by insulin have been developed to study diabetes and integrating this aspect in the model can bring a global picture of nutrition related diseases, as obesity is often linked to type II diabetes. Other hormones, such as gut hormones, could also be included in the model to strengthen the regulation at short-time scales.

In Chapter III, the regulation of food intake by leptin was considered to be mediated by a single type of leptin receptors, located in the hypothalamus. Brain leptin was thus implicitly considered to be proportional to plasma leptin. However, as we have seen in Chapter I, the ratio between plasma and CSF leptin is not constant and the transport of leptin from the plasma to the CSF is mediated by leptin receptors LRa located at the blood-brain barrier. So these receptors can impact the regulation of food intake by leptin, by modulating the transport of leptin from the blood to the CSF. Including the description of their dynamics in the model would probably improve the model, and result in explicitly considering brain leptin as the regulator of food intake and leptin receptors in the hypothalamus.

In this manuscript, food intake was supposed to be determined only relatively to physi-

ological needs: hormonal signals are integrated to determine the energy needed which is then consumed. We did not considered that food could be consumed when sated. However, palatable foods can induce a rewarding effect, involving dopamine release, and this can later induce an overconsumption without physiological requirement [Volkow et al., 2011]. Dopamine signalling is reduced in obesity, and is associated with compulsive food intake, in particular of high fat foods [Volkow et al., 2011]. Including this reward effect in our models would then improve the description of food intake regulation, and possible dysregulations leading to obesity.

Models presented in Chapter II, III and IV have been tested on experimental data on rats, for specific experiments (caloric restriction and leptin injection). It would be interesting to test the predictive aspects of the models against other data sets. First, considering other experimental conditions, such as overfeeding or high-fat diet would allow to study the reaction to an excess of calories on the dynamics of body weight, in particular regarding the adaptation of the rate of energy expenditure and the development of leptin resistance. Applying the model to other species, in particular humans, would bring an interesting input on the hormonal regulation of body weight in particular cases not considered in experiments and not accessible by actual models. For other rodent species, such as mice, the changes would not be important, as their body composition dynamics is similar to rats, as well as the hormonal mechanisms regulating food intake. More important changes would be necessary to apply the models to humans, as their body weight remains quite constant during adult life, unlike rodents. However, mechanisms regulating food intake, such as the effect of hormones ghrelin and leptin, are similar in humans and rodents. The main change would be a modification in the equations describing body composition. As humans control actively their food consumption to avoid changes in body weight, it could also be possible to include this control part in the model to represent a psychological control of food intake. However, it seems complicated to estimate the impact of this control.

In the models described in this thesis, adipose tissue was considered as an homogeneous entity, however, as we have seen in Chapter I adipose tissue is composed of adipocytes, which have specific dynamics. Adipocytes size distribution is bimodal and is modulated by the weight status of individuals (in particular obesity). The production of leptin by each adipocyte is function of its lipid content, which depends on the global state of the adipose tissue. It could then be relevant to couple the model of body weight and body composition dynamics to a model of adipocytes dynamics.

As we have seen in this thesis, mathematical modeling can bring helpful insight in the qual-

itative and quantitative study of the behavior of complex systems, such as the regulation of food intake and body weight, with only a limited experimental input. Our contribution, although limited to a small description of body weight regulation by hormones, highlighted key processes leading to the adaptation of the rate of energy expenditure and to the development of leptin resistance by efficiently combining mathematical and computational tools with biological experiments. The interdisciplinary nature of this work contributed to tackle scientific questions relevant for all disciplines involved in this work, hence stressing the important role of mathematical and computational biology in modern biology.

# Appendix A

# Analysis of a simplified system

## A.1 Model formulation

Let consider System (III.10):

$$\begin{cases} \dfrac{\mathrm{d}FM}{\mathrm{d}t} &= \dfrac{\gamma_E FI - \eta((\rho_{FM} + \rho_{FFM}\gamma_\Omega)FM + \rho_{FFM}\gamma_\Omega \alpha \exp(\kappa FM)/\kappa + \rho_{FFM}C + \xi)}{\rho_{FFM}\gamma_\Omega(1 + \alpha\exp(\kappa FM)) + \rho_{FM}}, \\ \dfrac{\mathrm{d}L}{\mathrm{d}t} &= \gamma_L FM - \delta_L L, \\ \dfrac{\mathrm{d}R}{\mathrm{d}t} &= \gamma_R(1 + \lambda_{R1}L) - \delta_R(1 + \lambda_{R2}L^2)R, \\ \dfrac{\mathrm{d}FI}{\mathrm{d}t} &= \dfrac{\gamma_{FI}(L^n + \theta^n)}{L^n(1 + \phi R) + \theta^n} - \delta_{FI} FI. \end{cases}$$

with all parameters being positive. Let's assume:

(H1) Variations of fat-free mass are negligible compared to variations of fat mass, so $dFFM/dFM = 0$. It follows that

$$\Omega := \gamma_\Omega(1 + \alpha\exp(\kappa FM)) = 0, \quad FFM = FFM_0,$$

and the first equation of System (III.10) becomes

$$\frac{dFM(t)}{dt} = \frac{\gamma_E FI(t) - \eta(\rho_{FM} FM(t) + \rho_{FFM} FFM_0 + \xi)}{\rho_{FM}}.$$

(H2) Following variations of the fat mass $FM$, leptin is instantaneously produced,

proportionally to $FM$, so that

$$L(t) = k_L FM(t), \quad \text{with } k_L = \frac{\gamma_L}{\delta_L},$$

Then, System (III.10) writes

$$\left\{ \begin{array}{rcl} \dfrac{dFM(t)}{dt} & = & \tilde{\gamma}_E FI(t) - \eta FM(t) - \nu, \\ \dfrac{dR(t)}{dt} & = & \gamma_R(1 + \tilde{\lambda}_{R,1} FM(t)) - \delta_R(1 + \tilde{\lambda}_{R,2} FM^2(t))R(t), \\ \dfrac{dFI(t)}{dt} & = & \dfrac{\gamma_{FI}}{1 + \phi_{R(t)}(k_L FM(t))} - \delta_{FI} FI(t), \end{array} \right. \tag{A.1}$$

with

$$\nu := \frac{\eta(\rho_{FFM} FFM_0 + \xi)}{\rho_{FM}},\ \tilde{\gamma}_E := \frac{\gamma_E}{\rho_{FM}},\ \tilde{\lambda}_{R,1} := \lambda_{R,1} k_L,\ \tilde{\lambda}_{R,2} := \lambda_{R,2} k_L^2.$$

For the sake of simplicity we will omit the tilde on parameter notations in the following.

We make the following additional assumptions:

(H3) Food intake is quasi constant (quasi steady state assumption) with $dFI(t)/dt = 0$ and

$$FI(t) = \frac{k_{FI}}{1 + \phi_{R(t)}(k_L FM(t))}, \quad k_{FI} := \frac{\gamma_{FI}}{\delta_{FI}} > 0.$$

(H4) The function $\phi_R(\cdot)$ is constant, with $\phi_R(L) = \phi R$.

Hence, System (A.1) becomes

$$\left\{ \begin{array}{rcl} \dfrac{dFM(t)}{dt} & = & \gamma_E \dfrac{k_{FI}}{1 + \phi R(t)} - \eta FM(t) - \nu, \\ \dfrac{dR(t)}{dt} & = & \gamma_R(1 + \lambda_{R,1} FM(t)) - \delta_R(1 + \lambda_{R,2} FM^2(t))R(t). \end{array} \right. \tag{A.2}$$

Assuming $\lambda_{R,1} \gg \lambda_{R,2}$, then a rescaling of System (A.2) gives (see Section A.3 for details)

$$\left\{ \begin{array}{rcl} \dfrac{dFM(t)}{dt} & = & \dfrac{\gamma_{FM}}{1 + \phi R(t)} - \eta FM(t) - \nu, \\ \dfrac{dR(t)}{dt} & = & \rho FM(t) - \delta_R(1 + \lambda_R FM^2(t))R(t), \end{array} \right. \tag{A.3}$$

where notations have been slightly modified, for the sake of simplicity. If $\lambda_{R1} = 0$, then System (A.2) can be directly analyzed.

## A.2 Model analysis

Let search for steady states $(m, r)$ of System (A.3). They are constant solutions, satisfying

$$\frac{\gamma_{FM}}{1+\phi r} = \eta m + \nu \qquad \text{and} \qquad r = \frac{\rho m}{\delta_R(1+\lambda_R m^2)}. \tag{A.4}$$

Using the notation,

$$\sigma := \frac{\rho}{\delta_R},$$

it follows, from (A.4),

$$\eta m + \nu = \gamma_{FM} \frac{1+\lambda_R m^2}{1+\phi\sigma m + \lambda_R m^2}.$$

Let define the functions $f_1$ and $f_2$ as follows:

$$f_1(x) := \frac{\eta}{\gamma_{FM}} x + \frac{\nu}{\gamma_{FM}}, \tag{A.5}$$

and

$$f_2(x) := \frac{1+\lambda_R x^2}{1+\mu x + \lambda_R x^2}, \quad \mu := \phi\sigma. \tag{A.6}$$

Let's focus on the function $f_2$. It equals 1 when $x = 0$ and $x = +\infty$. Moreover,

$$f_2'(x) = \mu \frac{\lambda_R x^2 - 1}{(1+\mu x + \lambda_R x^2)^2}$$

so $f_2$ is decreasing for $x < x_1$ and increasing for $x > x_1$, where $x_1 := \sqrt{1/\lambda_R}$, with

$$f_2(x_1) = \frac{2\sqrt{\lambda_R}}{2\sqrt{\lambda_R} + \mu}.$$

In addition,

$$f_2''(x) = 2\mu \frac{\mu + 3\lambda_R x - \lambda_R^2 x^3}{(1+\mu x + \lambda_R x^2)^3}$$

so there exists a unique $x_2 > 0$ such that $f_2''(x_2) = 0$, with $0 < x_1 < x_2$ (one can easily check that $x_2 > \sqrt{3/\lambda_R}$). Hence, the function $f_2$ is positive, decreasing on the interval $[0, x_1]$, increasing on the interval $[x_1, +\infty)$, convex on the interval $[0, x_2]$ and concave for $x > x_2$. We then reach the following conclusion (see Figure A.1):

**Proposition 1.** *Depending on the value of $\gamma_{FM}$, the problem $f_1(x) = f_2(x)$ may have 0, 1, or 3 solutions, which correspond to steady states $(m, r)$ of System* (A.3)*:*

**Case 1** *If $\gamma_{FM} < \nu$, then System* (A.3) *has no steady state;*

**Case 2** *If $\gamma_{FM} \geq \nu$, and $\nu/\gamma_{FM} \approx 1$, then System* (A.3) *has only one steady state, with*

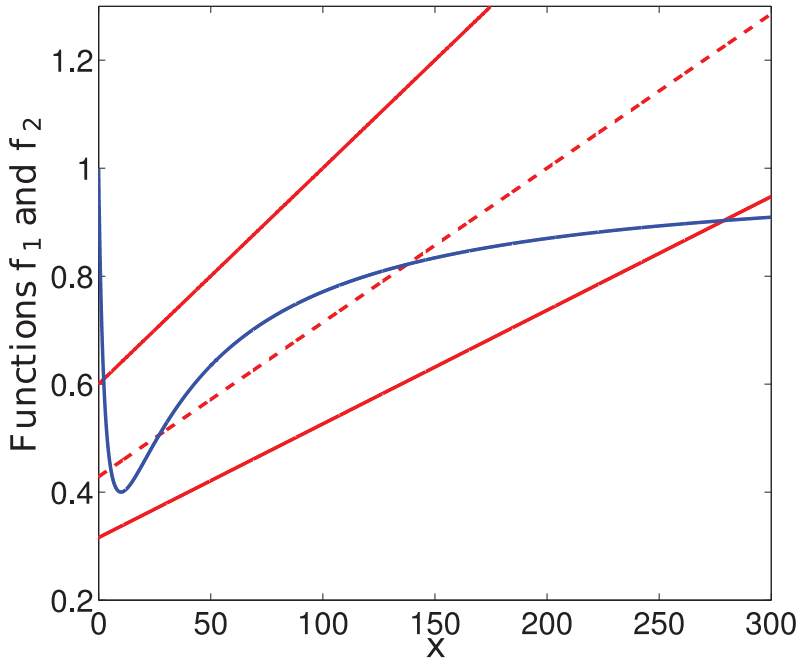


**Figure A.1** – Graphs of the functions $f_1$ and $f_2$ defined in (A.5) and (A.6) for three different values of $\gamma_{FM}$, all satisfying $\gamma_{FM} > \nu$. The graph of function $f_2$ is displayed in blue, whereas functions $f_1$ are displayed in red. The top red line corresponds to $\gamma_{FM} = 50$ and $\nu/\gamma_{FM} = 0.7$, there is only one intersection between the two curves in the vicinity of $x = 0$. The same occurs for the bottom red curve, corresponding to $\gamma_{FM} = 95$, and the intersection occurs for $x$ large. The middle red dashed curve corresponds to $\nu/\gamma_{FM} = 0.7$, and is associated with three intersections (see Proposition 1).

$(m, r) \approx (0, 0)$;

**Case 3** *If $\gamma_{FM} \geq \nu$, and $\gamma_{FM}$ is large, then System* (A.3) *has only one steady state, with* $(m, r) \approx (+\infty, 0)$;

**Case 4** *If $\gamma_{FM} \geq \nu$, with $\gamma_{FM} \in (\gamma_{min}, \gamma_{max})$ (values $\gamma_{min}$ and $\gamma_{max}$ are to be determined and depend on other parameter values), then System* (A.3) *has 3 steady states, denoted by $(m_l, r_l)$, $(m_m, r_m)$, and $(m_h, r_h)$ respectively, such that $m_l < m_m < m_h$, $f_1'(m_l) > f_2'(m_l)$, $f_1'(m_m) < f_2'(m_m)$, and $f_1'(m_h) > f_2'(m_h)$.*

*Proof.* Here are some hints for each case. Case 1: the function $f_1$ is an increasing function satisfying $f_1(0) > 1 \geq f_2(x)$ for all $x \geq 0$. Case 2: The function $f_1$ becomes larger than 1 for $x$ close to 0 so the problem $f_1(x) = f_2(x)$ has only one solution ($f_1$ is increasing, $f_2$ is decreasing) in the vicinity of $x = 0$. Case 3: The function $f_1$ is flatter, yet goes towards infinity, and then crosses the bounded function $f_2$ for large values of $x$. Case 4: This case is illustrated on Figure A.1. □

Let's now focus on the linear stability of the steady states of System (A.3). Let denote by $(m, r)$ a steady state of (A.3). Linearization of (A.3) around $(m, r)$ leads to

$$\begin{cases} \dfrac{dFM(t)}{dt} &= -a(r)R(t) - \eta FM(t), \\ \dfrac{dR(t)}{dt} &= b(m,r)FM(t) - c(m)R(t), \end{cases}$$

where

$$\begin{aligned} a(r) &= \frac{\gamma_{FM}\phi}{(1+\phi r)^2} &> 0, \\ b(m,r) &= \rho - 2\lambda_R\delta_R mr, & \\ c(m) &= \delta_R(1+\lambda_R m^2) &> 0. \end{aligned}$$

Hence, $(m, r)$ is locally asymptotically stable if and only if

$$-\eta - c(m) < 0$$

and

$$\eta c(m) + a(r)b(m,r) > 0.$$

The first condition is straightforwardly satisfied due to the positivity of $c(m)$. The second condition writes

$$\eta\delta_R(1+\lambda_R m^2) + \frac{\gamma_{FM}\phi}{(1+\phi r)^2}(\rho - 2\lambda_R\delta_R mr) > 0. \tag{A.7}$$

Using (A.4), Inequality (A.7) is equivalent to

$$H(m) > 0,$$

where

$$H(m) := \eta(1+\mu m+\lambda_R m^2)^2 - \gamma_{FM}\mu(\lambda_R m^2 - 1).$$

It follows that $H(m) > 0$ if and only if

$$f_2'(m) < \frac{\eta}{\gamma_{FM}} = f_1'(m). \tag{A.8}$$

We can then conclude to the stability of System (A.3) in the next Proposition.

**Proposition 2.** *When System* (A.3) *has only one steady state, then it is locally asymptotically stable. When System* (A.3) *has 3 steady states, then the steady states* $(m_l, r_l)$ *and* $(m_h, r_h)$*, associated with the lower and higher values of* $m$*, respectively (see Proposition 1), are locally asymptotically stable whereas the intermediate steady state* $(m_m, r_m)$

*is unstable: System* (A.3) *is bistable.*

## A.3 Rescaling of system (A.2)

Consider System (A.2), and set

$$m(t) := \frac{\lambda_{R,1} FM(t) + 1}{\sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}}, \qquad r(t) := R(t).$$

Then $m$ and $r$ satisfy

$$\begin{cases} m'(t) &= \dfrac{\gamma_m}{1+\phi r(t)} - \eta m(t) - \nu_m, \\ r'(t) &= \gamma_r m(t) - \delta_r \left( 1 - 2\dfrac{\lambda_{R,2}}{\sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}} m(t) + \lambda_{R,2} m^2(t) \right) r(t), \end{cases}$$

where

$$\gamma_m := \gamma_E k_{FI} \frac{\lambda_{R,1}}{\sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}}, \qquad \nu_m := \frac{\lambda_{R,1}\nu - \eta}{\sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}},$$
$$\gamma_r := \gamma_R \sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}, \qquad \delta_r := \delta_R \frac{\lambda_{R,1}^2 + \lambda_{R,2}}{\lambda_{R,1}^2}.$$

Since we assumed $\lambda_{R,1} \gg \lambda_{R,2}$, then

$$\frac{\lambda_{R,2}}{\sqrt{\lambda_{R,1}^2 + \lambda_{R,2}}} \approx 0$$

and

$$\gamma_m \approx \gamma_E k_{FI} = \gamma_{FM}, \qquad \nu_m \approx \nu - \frac{\eta}{\lambda_{R,1}},$$
$$\gamma_r \approx \gamma_R \lambda_{R,1}, \qquad \delta_r \approx \delta_R.$$

One obtains System (A.3).

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