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A model for regulated fatty acid metabolism in liver; equilibria and their changes

Abstract. We build a model for the hepatic fatty acid metabolism and its metabolic and genetic regulations. The model has two functioning modes: synthesis and oxidation of fatty acids. We provide a sufficient condition (the strong lipolytic condition) for the uniqueness of its equilibrium. Under this condition, modifications of the glucose input produce equilibrium shifts, which are gradual changes from one functioning mode to the other. We also discuss the concentration variations of various metabolites during equilibrium shifts. The model can explain a certain amount of experimental observations, assess the role of poly-unsaturated fatty acids in genetic regulation, and predict the behavior of mutants. The analysis of the model is based on block elimination of variables and uses a modular decomposition of the system dictated by mathematical global univalence conditions.

Introduction

Metabolic and genetic model of fatty acid metabolism. Recent advances in genetics and in physiology show the necessity for melting together several types of cultures in order to understand animal and human nutrition and solve important health and economic problems. Metabolic analysis approaches study dynamics of biochemical pathways and employs detailed knowledge of biochemical reactions mechanisms [Men97, Fel97, HS96, CB95, MLR90, PSP+04, PRP+03]. Genetic functional studies mostly concern gene networks, recently integrating some metabolites [dJGH+04, CCRFS05, LSS+05]. Gene network dynamics is modeled by various methods: systems of differential equations [TCN03], boolean or multivalued logical automata [Kau93, ST01], Petri nets [CRRT04, MDNM00]. Although virtual cell models are planned by many, present studies deal with simple cell functions. At a higher level of complexity, integrative approaches were applied to modeling various organs, the heart being one of the best studied [Nob02]. The goal of these studies is to explain physiology from molecular basis. The two main obstacles against this goal are the sparseness of the biological knowledge and the mathematical difficulty of analyzing large complex systems.

Main pathways of carbohydrate metabolism are considered to be the cornerstones of metabolic modeling [ECB98, Cha02, TPa00]. However, models of metabolism in eukaryotes are scarce and dedicated to specific metabolic pathways and organs [LK02]. Furthermore, although recent experiments pointed out the genetical changes induced by diet in various organisms [CWM05, ACW+03, La04, BLC+04], metabolic dynamical modeling is rarely considering the genetic context. Concerning mathematical complexity, one can reduce the number of numerical parameters in the models by building so-called "minimal models" [BIBC79, VAS+02, CWS04, TCB+06]. Even for such minimal models inferring parameters from data posses non-trivial problems [MSBC06].

In this paper we present a mixed metabolic and genetic model of regulated fatty acids metabolism in liver, representing a reasonable compromise among these various cultures. Parallel work by [Bel05] on liver focus on transport processes and do not discuss genetic regulation. The importance of genetic

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regulation in fatty acid metabolism was recently emphasized [CF03, PLM03, DF04, Jum04]. Here we trade off the complexity of spatial effects against the complexity of regulations and the possibility of analytical reasonings. Our analysis of the model do not use numerical parameters. It puts forward a mathematical qualitative approach that can be used more generally for the analysis of equilibria of complex biological systems.

Questions raised by mixed models. Melting cultures generates many, conceptually diverse, questions. Let us point out three such questions related to multistability, timescales and the role of genetic and metabolic regulations.

Mixed genetical and metabolic systems are often multistable. As a well known illustration, the functioning of the E.Coli lactose operon is based on bistability [PJM59, YM03]. Thus, the change in food (lactose) induces an equilibrium switch which represents a jump from one attractor to another. Equilibrium switch is efficient in saving resources (enzymes), because these are produced only on demand. As a counterpart it is less flexible and tuning is not possible (the response is of the binary type).

The alternative way to adapt to external changes is via equilibrium shifts. Then, during an equilibrium shift, an equilibrium uniqueness condition is fulfilled and there are no jumps between attractors. The jumps are replaced by smooth, gradual changes.

Fatty acid metabolism in hepatocytes has two antagonistic functioning modes which could suggest bistability: synthesis produces fat reserves; lipolysis burns fats and produces energy. This motivates the first question we wish to answer: in higher organisms, does the whole of regulations produce multistationarity or a unique equilibrium of fatty acid metabolism? We shall argue that during a change in food, a unique equilibrium shifts smoothly between the two functioning modes and that there is no bistability.

The second question we wish to answer is about timescales. Genes coding for enzymes need relatively long times to change expression levels and enzymes concentrations. On short time scales enzyme concentrations can be considered to be constants. This suggests that changes of nutritional conditions induces processes with various timescales. We want to know whether there are any physiological consequences of the multiple timescales, and to find simple ways to take this into account in our modeling. For instance, fasting demands a shift from synthesis to lipolysis functioning modes. This can be done rapidly by metabolic control. Genetic regulation brings slower changes that push the shift further. Other slow processes (for instance diffusion-controlled lipid transport within different organs) could be responsible for other long time scales. We shall limit our analysis to only two timescales: a metabolic, fast one, and a genetic, slow one.

Among the fatty acids, many work describe the interference between genes and a special class of fatty acids (polyunsaturated fatty acids denoted by PUFA) [Jum04]. PUFA are synthetized from essential fatty acids, that are taken from the diet. To the contrary, saturated and mono-unsaturated fatty acids (denoted by S/MU-FA) are synthesized de novo in liver. PUFA control their own oxidation as well as the synthesis and oxidation of the other fatty acids. The control is due to formation of complexes that activate or inhibit the active forms of nuclear receptors regulating the transcription of genes coding for enzymes involved in the corresponding pathways.

The third question we wish to answer is about the effects of the PUFA interaction with genes, within normal and mutant genotypes. We shall argue that, when genetic regulations are absent (for instance, in PPAR-knocked out cells), the increase of PUFA concentration during fasting is stronger than in wild type cells.

Main points of the paper. The paper is structured as follows.

- *Definition of several classes of partial equilibria associated with a mixed model.* In Section 1, we introduce mixed models, that is, a differential system of equations where genetic/slow variables and metabolic/fast variables are distinguished. Associated to this model, we derive two types of partial equilibria: first, the well-known *quasi-stationnarity* or *non-genetically regulated equilibrium*, where genetic variables are supposed to be constant. Second, *genetic partial equilibrium* where genetic variables are supposed to be at equilibrium. Partial equilibria result from a reduction method consisting in successive elimination of variables of the model.
- *Construction of a mixed differential model.* In Section 2 we build a differential model for the regulated fatty acid metabolism in liver. In order to reduce complexity, we deliberately choose a

simplified description of the main metabolic pathways and of the genetic regulations. Although caricatural, the models exhibits the regulatory function of PUFA and agrees with recent experiments. Our model is quite general since we do not use explicit forms of the functions relating metabolic fluxes to genetic or metabolic variables. We just take into account the signs of the variations of elementary fluxes with respect to the variables. Equilibrium equations allow to extract implicit relations between variables. This approach is close to metabolic control [CB95, Fel97, HS96] and also to classical equilibrium thermodynamics [Cal05].

– **Study of equilibria.** In Section 3 the steady states of the differential model are studied. We find a sufficient condition for the uniqueness of equilibrium. This condition is expressed mathematically as an inequality involving partial derivatives of the fluxes with respect to metabolites (elasticities). We discuss a biological interpretation of this condition.

– **Qualitative validation, prediction and illustration.** In Section 4 we develop the qualitative analysis of the model. The behavior of the model is coherent with known experimental data. Moreover, the model has several predictions concerning the effect of suppressing the genetic regulation of the important nuclear factor PPAR-α, also on the role of genetic regulation for energy recovering at fasting.

Using standard regulation functions, we propose an explicit set of differential equations which represents the dynamics of hepatic fatty acid metabolism and its regulations. We give numerical simulations which illustrate the main results of the paper.

– **Discussion.** Section 5 is devoted to a discussion of the results and of possible extensions.

– **Mathematical method.** Section 6 provides details about the mathematical method. This method uses a decomposition of the system into boxes or modules that are chosen in order to fulfil the conditions of a global univalence theorem (Gale-Nikaido).

1. Mathematical framework: mixed model; associated partial equilibria states

Mixed metabolic-genetic, slow-fast decomposition. Main carbohydrate metabolic pathways are rather well documented [Sal99]. Nevertheless, actual models of these metabolic pathways do not take into account genetic regulation. In these models [Cha02] enzyme concentrations are parameters, rather than dynamical variables. We introduce here a mixed metabolic/genetic system that contains metabolites, proteins (products of genes), especially transcription factors and enzymes, as dynamical variables.

Our mixed model for genetically regulated metabolism is represented by a system of differential equations:

$$(\mathcal{S}) : \begin{cases} \frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) \\ \frac{d\mathbf{Y}}{dt} = \mathbf{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}). \end{cases} \tag{1.1}$$

where $\mathbf{p} \in \Delta$, Δ being a compact subset of $\mathbb{R}^q$, stands for a set of external parameters. The dynamical variables are partitioned into two groups. Concentrations of metabolites involved in biochemical reactions are metabolic variables, represented by the vector $\mathbf{X} \in \mathbb{R}^n_+$. Concentrations of proteins (basically enzymes and transcription factors) are genetic variables, represented by the vector $\mathbf{Y} \in \mathbb{R}^m_+$.

Most of the genetic variables vary on timescales generally much longer than any of the metabolic variables: genetic variables including concentration of products of genes (enzymes, transcription factors) have significant variations on long (genetic) time scales τ_G. On short, metabolic, time scales $\tau_M << \tau_G$ these variables can be considered to be fixed. Thus, our partition of the variables corresponds to the well known slow-fast decomposition of dynamical systems [Mur03].

In Eq.(1.1), $\mathbf{\Phi}$ is the time derivative of fast (metabolic) variables and $\mathbf{\Psi}$ is the time derivative of slow (genetic) variables. Beyond timescales, there is a physical difference between these functions. $\mathbf{\Phi}$ is a combination of generally conservative, metabolic fluxes. $\mathbf{\Psi}$ have no reason to be conservative. The consequences of this difference will show up in the construction of the model (in particular when identifying the relations among fluxes).

Equilibrium and quasi-stationary states. Equilibria are defined mathematically as fixed points of a system of differential equations and biologically as *stationary states* in which measurable macroscopic quantities stop changing.

Given the parameter $\mathbf{p}$, recall that an *equilibrium state* of the system (1.1) is defined by the following equations:

$$\begin{cases} \frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0 \\ \frac{d\mathbf{Y}}{dt} = \mathbf{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0. \end{cases} \tag{1.2}$$

Given a decomposition of variables and a parameter $\mathbf{p}$, we can define another notion of equilibrium, called *quasi-stationary state*, which is the equilibrium state of the subsystem associated to the variable $\mathbf{X}$, constrained by fixing $\mathbf{Y}$. A quasi-stationary state satisfies:

$$\begin{cases} \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0 \\ \mathbf{Y} = \mathbf{Y}_0 (= \text{const.}). \end{cases} \tag{1.3}$$

As detailed before, mixed metabolic-genetic systems are slow-fast systems. On metabolic, time scales $\tau_M << \tau_G$ genetic variables can be considered to be fixed. Therefore, within metabolic timescale τ_M the system reaches only quasi-stationarity. After a longer time of the order τ_G, it reaches equilibrium.

Slow-fast dynamics; genetically non-regulated trajectory. The slow manifold of a slow-fast system is defined by the equations $\mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$.

Although the definition of a quasi-stationary state (Eq.(1.3)) can always be used, this state has a dynamical meaning only within some conditions which are those required for the applicability of the Tikhonov theorem [TVS80,Was65] or of the geometrical theory of Fenichel of singular perturbations [Fen79]. Indeed, the slow manifold should be hyperbolically stable with respect to the constrained dynamics $\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}_0, \mathbf{p})$ (see Section 5 for details).

Under this condition, trajectories of slow-fast systems are made of two parts: a rapid part finishing close to the slow manifold and along which $\mathbf{Y}$ is practically constant and a slow part practically included in the slow manifold [Was65,Fen79,Mur03].

Let us call *genetically non-regulated trajectory* the set of points approximating the rapid part of the slow-fast trajectory, that is, following the dynamics $\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}_0, \mathbf{p})$ and $\mathbf{Y} = \mathbf{Y}_0$. The quasi-stationary state is the intersection of the genetically non-regulated trajectory and the slow manifold (see figure 1.1). It represents the natural intermediate stage on the way towards equilibrium.

Genetically non-regulated model. By definition, metabolic variables on the genetically non-regulated trajectory are governed by a differential dynamical system for metabolic variables that we call *genetically non-regulated model*:

$$\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}_{gnr}(\mathbf{X}, \mathbf{p}) \tag{1.4}$$

where $\mathbf{\Phi}_{gnr}(\mathbf{X}, \mathbf{p}) = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}_0, \mathbf{p})$ are called *reduced fluxes*.

Moreover, on the genetically non-regulated trajectory, the quasi-stationary equation (1.3) reduces to the following equations for the metabolic variables, that are called *genetically non-regulated state equations*:

$$\mathbf{\Phi}_{gnr}(\mathbf{X}, \mathbf{p}) = 0 \tag{1.5}$$

Let us note that the genetically non-regulated model is the playground for classical metabolic analysis. Indeed, the vast majority of metabolic analysis studies do not take into account genetic regulations and consider that the concentrations of enzymes are fixed.

Genetic partial equilibria. According to the classical singular perturbation theory, the constraint $\mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$ allows expressing the fast (metabolic) variables $\mathbf{X}$ as functions of the slow (genetic) variables $\mathbf{Y}$. In this paper we perform something different. We have already introduced the quasi-stationarity states and the equilibrium states. Let us introduce another type of intermediate states obtained by equilibrating only the genetic variables. Let us call these states *genetic partial equilibria*. Contrary to quasi-stationay states (that are natural intermediate stages on the way towards equilibrium), genetic partial equilibria are not dynamically reachable (because genetic variables equilibrate after and not before the metabolic ones). However, they represent mathematical constructions useful for the study of equilibria.

More precisely, we intend to express genetic variables as functions of the metabolic variables thanks to the genetic partial equilibrium equation $\mathbf{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$. The following condition ensures that these equations have solutions in variables $\mathbf{Y}$.

Condition 1 (Unique genetic partial equilibrium)
The genetic partial equilibrium equations $\mathbf{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$ have a unique solution in $\mathbf{Y}$ which is a smooth function of $\mathbf{X}$ for all $\mathbf{X} \in \mathbb{R}_+^n, p \in \Delta$. In other words, genetic null-clines are smooth and have unique intersections with the hyperplanes $\mathbf{X} = const.$.
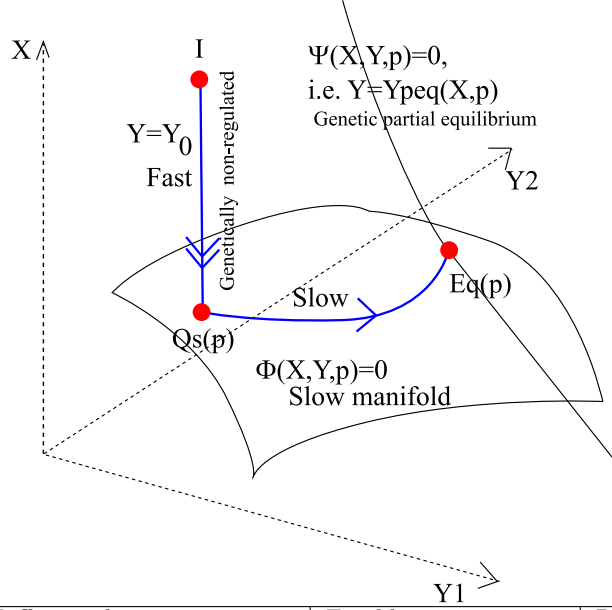
Model [Variables]	Differential equations	Equilibrium equations	Relation with the original model
Mixed differential model $[\mathbf{X}, \mathbf{Y}]$	$\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p})$ $\frac{d\mathbf{Y}}{dt} = \boldsymbol{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}).$	$\boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$ $\boldsymbol{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0.$	
genetically non-regulated model $[\mathbf{X}]$	$\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}_{gnr}(\mathbf{X}, \mathbf{p})$ $= \boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}_0, \mathbf{p})$	$\boldsymbol{\Phi}_{gnr}(\mathbf{X}, \mathbf{p}) = 0$	Fast dynamics. Equilibria correspond to quasi-stationnarity of the mixed model.
genetic partial equilibrium model $[\mathbf{X}]$	$\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p})$ $= \boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}), \mathbf{p})$ $\boldsymbol{\Psi}(\mathbf{X}, \mathbf{Y}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}), \mathbf{p}) = 0$	$\boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}) = 0$	No dynamic approximation of the full system. Equilibria correspond to equilibrium states of the mixed model.

Fig. 1.1. Geometry of the mixed decomposition. For illustration we considered one metabolic variable $\mathbf{X} = (X)$ and two genetic variables $\mathbf{Y} = (Y_1, Y_2)$. The slow manifold and the genetic null-cline have the equations $\boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$, and $\boldsymbol{\Psi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$, respectively. The slow manifold is considered hyperbolically stable with respect to the constrained dynamics $\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}_0, \mathbf{p})$. Trajectories starting from the initial state I have a rapid part on which genetic variables are constant $\mathbf{Y} = \mathbf{Y}_0$ and a slow part in the slow manifold. The quasi-stationary state $Qs(p)$ is the intersection of the slow manifold with the line $\mathbf{Y} = \mathbf{Y}_0$ of genetically non-regulated states. The equilibrium state $Eq(p)$ is the intersection of the slow manifold with the genetic null-cline $\mathbf{Y} = \mathbf{Y}_{peq}(\mathbf{X}, \mathbf{p})$ which is the line of genetic partial equilibrium states.

If Condition 1 is satisfied, we denote the corresponding implicit functions $\mathbf{Y} = \mathbf{Y}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p})$. This equation defines the *genetic null-cline* (see Fig.1.1), that is, the trajectory of the dynamics $\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p})$, $\mathbf{Y} = \mathbf{Y}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p})$ where $\boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}) = \boldsymbol{\Phi}(\mathbf{X}, \mathbf{Y}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}), \mathbf{p})$ are called *reduced fluxes*.

On the genetic null-cline, we can simply express the equilibrium equations (1.2) as a set of constraints for the metabolic variables only. In analogy to classical thermodynamics [Cal05] we call these reduced constraints *genetic partial equilibrium state equations* for metabolic variables:

$$\boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}) = 0 \tag{1.6}$$

The vector field $\boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p})$ defines itself a differential dynamical system governing the metabolic variables on the genetic nullcline. We call this system the *genetic partial equilibrium model*:

$$\frac{d\mathbf{X}}{dt} = \boldsymbol{\Phi}_{\mathrm{peq}}(\mathbf{X}, \mathbf{p}). \tag{1.7}$$

Equilibria and equilibria shifts. Our purpose is to discuss equilibria and equilibria shifts of the full system. For this purpose, the partial equilibrium model is fully suitable, as follows from:

5

Proposition 1.1. *The equilibria of the partial equilibrium model (Eq.(1.7)), i.e. the solutions of the genetic partial equilibrium state equations (1.6) are the equilibria of the full system (1.1).*

The equilibria of the genetically non-regulated model (Eq.(1.4)), i.e. the solutions of the genetically non-regulated state equations (1.5) are the quasi-stationary states of the full system (1.1).

State equations help us to assess the uniqueness or the multiplicity of equilibria, or to estimate the variations of the metabolites when the external parameters change. Furthermore, they allow to extend notions from the control theory of metabolism such as elasticities [CB95] to the case when genetic regulation is present. The essential of metabolic control is expressed by the following formula which is a consequence of the implicit function theorem:

$$\frac{d\mathbf{X}}{d\mathbf{p}} = -\left[\frac{d\mathbf{\Phi}_{red}}{d\mathbf{X}}\right]^{-1} \frac{d\mathbf{\Phi}_{red}}{d\mathbf{p}} \qquad (1.8)$$

Eq.(1.8) says that the response of a metabolic system to changes of the parameters can be calculated from the state equation. The choice for the function $\mathbf{\Phi}_{red}$ should be either $\mathbf{\Phi}_{gnr}$ or $\mathbf{\Phi}_{peq}$ depending on the timescale of the changes. If changes are monitored on short, metabolic timescales then one should consider genetically non-regulated state equations. If the changes are monitored on long, genetic timescales then one should consider partial equilibrium state equations. In all cases, it is important to compute the derivative matrix $\frac{d\mathbf{\Phi}_{red}}{d\mathbf{X}}$. This matrix describes the resistance of the metabolic variables to forcings and is analogous to the matrix of elastic constants in elasticity theory [CB95]. $\frac{d\mathbf{\Phi}_{gnr}}{d\mathbf{X}}$ gives the instantaneous elastic constants, while $\frac{d\mathbf{\Phi}_{peq}}{d\mathbf{X}}$ gives the static elastic constants (resistance to slow, adiabatic changes, taking into account genetic readjustment).

Comments.

– If Condition 1 is not satisfied then there are several equilibrium state equations in the metabolic state variables. We shall not discuss this situation in this paper.
– This particular type of reduction implies a certain emphasis on metabolic variables. It is justified when genetic variables are not measured or when we are mainly interested in variations of metabolites.
– The genetic partial equilibrium model represents a bad approximation for the dynamics of the full system. On the contrary, the genetically non-regulated model represents a good approximation for the rapid part of the trajectories of the full dynamical system Eq.(1.1) (see Fig.1.1). The correct approximation of the slow parts of the trajectories is given by the slow/fast decomposition, more precisely by the reduced system $\frac{d\mathbf{Y}}{dt} = \mathbf{\Psi}(\mathbf{X}_{red}(\mathbf{Y}, \mathbf{p}), \mathbf{Y}, \mathbf{p})$, $\mathbf{X} = \mathbf{X}_{red}(\mathbf{Y}, \mathbf{p})$ where $\mathbf{\Phi}(\mathbf{X}_{red}(\mathbf{Y}, \mathbf{p}), \mathbf{Y}, \mathbf{p}) = 0$.

2. Mixed model for genetically regulated fatty acid metabolism

We provide here the details of our model and we derive the associated genetically non regulated and genetic partial equilibrium models.

In different species such as chicken, rodents and humans, hepatocyte (liver) cells have the specificity to ensure both lipogenesis and β-oxidation. To set ideas, all the variables of the model pertain to an "abstract" hepatocyte, capable of the two different functioning modes.

2.1. Variables and fluxes for the mixed model of regulated fatty acid metabolism

Metabolic variables.

We have selected the most important metabolites implied in the fatty acid metabolism in liver as follows. Corresponding symbols for these variables are given in Table 2.1.

– ***Acetyl-CoA*** generated in mitocondria is the first brick for building fatty acids. It is consumed in lipogenesis in hepatocytes, produced in oxidation.
– ***Saturated and monounsaturated fatty acids*** (denoted by ***S/MU-FA***) can be produced by the organisms from Acetyl-CoA. They can also enter the metabolism as part of the diet.
– ***Exogenous polyunsaturated fatty acids (PUFA)*** are implied in genetic regulations. They can be manufactured from essential fatty acids which can not be produced by animals. As a simplification, we write that PUFA can only enter the metabolism as part of the diet.

– **_Energy (ATP)_** expresses the energy that the cell has at its disposal.

Notice two fundamental points: first, we have introduced the level of energy of the cell as a variable. Second, we have divided fatty acids into two parts: the ones that are implied in the genetic regulation, that is PUFA, and the ones that are not. PUFA are synthetized for essential fatty acids provided by the diet; hence we consider that this class can not be produced by the cell. Even if simplified, this distinction will allow us to model better the regulations of the metabolism.

Parameter. The system is driven by the glucose concentration, representing food. Different nutritional states such as normal feeding or fasting are modeled by different values of this parameter.

Primitive metabolic fluxes. Main metabolic processes are modeled here as *primitive fluxes*. They are represented as unstructured reactions, whose detailed mechanisms are not described. The corresponding symbols are given in Table 2.1.

– **_Glycolysis_** (in which we include the Pyruvate dehydrogenation reaction) produces Acetyl-CoA from glucose. Glycolysis can be considered reversible. Nevertheless, we shall not study glucose dynamics (G is a constant); reversibility will be neither used nor rejected.
– **_Krebs cycle_** produces energy for cellular needs from Acetyl-CoA.
– **_Ketone bodies exit_** allows the cell to to transfer the energy stored in Acetyl-CoA to the outside; it represents an important source of survival during fasting or starving.
– **_Lipogenesis_** transforms Acetyl-CoA first into citrate, then into saturated and monounsaturated fatty acids S/MU-FA.
– A **_outtake flux_** allow S/MU-FA to exit liver and go to storing tissues (adipocytes). Conversely, the intake flux is fed partially from diet, partially from lipolysed adipocytes. The intake flux is conventionally considered positive.
– Similar **_intake/outtake flux of PUFA_** allows PUFA to enter or exit the cell. Above diet and lipolysis, the intake flux of PUFA also includes a synthetic pathway consisting of desaturation and elongation of essential fatty acids.
– **_β−oxidation_** burns all fatty acids in order to produce energy and to recover Acetyl-CoA.
– ATP consumption expresses the energy (ATP) the cell consumes for living.
– **_Degradation of metabolites_** (Acetyl Co-A, S/MU-FA, PUFA) is used with a broad meaning including cell growth induced dilution, leaks or transfers to non-represented pathways, and effective molecular degradation. Negligible on the timescale of the metabolic processes, these processes can not be neglected on the genetic timescale.

Functioning modes. There are two functioning antagonist modes of the fatty acid metabolism in liver: lipogenesis that produce reserves, fatty acid oxidation that burns reserves and produces energy. The choice of the functioning mode depends on nutrition conditions: a lack of food (i.e. a sustained low level of glucose) stimulates lipolysis and oxidation; normal feed (normal glucose level) induces lipogenesis.

Fatty acids and genetic control. A good part of the known regulation mechanisms implies transcription factors such as nuclear receptors PPARα (peroxisome proliferator activated receptor α) and LXRα (liver X receptor α). The latter is known to activate the transcription of SREBP-1 (Sterol response element binding protein 1) known to trans-activate different genes involved in fatty acids synthesis and desaturation.

Concerning the metabolites, it has been established that fatty acids can up-regulate or down-regulate the expression of different genes controlling their metabolism. The regulatory effect is mainly due to PUFA: the interaction of S/MU-FA with genes is supposed weak. More precisely, it has been proposed that PUFA regulates the activity of SREBP-1 and of several members of the steroid-thyroid superfamily of nuclear receptors such as PPARα and LXRα (for reviews see [CF03,PLM03,DF04,Jum04]).

Genetic variables. Since the genetic interactions between metabolites and fluxes is not direct, we consider the following abstractions for the genetic regulation variables whose corresponding symbols are given in Table 2.1:

– the active form of the nuclear receptor PPAR,
– the active form of the nuclear receptor LXR, representing in a very simplified way the regulation path LXRα-SREBP-1.

– a representative abstract enzyme for each set of enzymes that are involved in S/MU-FA synthesis and oxidation, PUFA oxidation and ketone bodies exit respectively. Abstract enzymes production is controlled by LXR (modelling the LXRα-SREBP-1 pathway) and PPAR.

Variable (Concentration)	Symbol	$\frac{d\,\text{product}}{dt}$
Acetyl Co-A	A	Φ_A
Saturated and monounsaturated fatty acids (S/MU-FA)	F_1	Φ_{F_1}
Poly-unsaturated fatty acids (PUFA)	F_2	Φ_{F_2}
Energy ATP	T	Φ_T
Active form of PPAR	PP	Ψ_1
Active form of the regulation path LXR-SREBP	L	Ψ_2
Enzymes of S/MU-FA synthesis	E_1	Ψ_3
Enzymes of S/MU-FA oxidation	E_2	Ψ_4
Enzymes of PUFA oxidation	E_3	Ψ_5
Enzymes of Ketone body exit	E_4	Ψ_6

Primitive flux	Symbol
Glycolysis	Gly
Krebs cycle	Krebs
Ketone bodies exit	Kout
Lipogenesis	Syn
$\beta-$oxidation of S/MU-FA	Oxi1
$\beta-$oxidation of PUFA	Oxi2
S/MU-FA fatty acids intake/outake	Fin1
PUFA fatty acids intake/outake	Fin2
ATP consumption	DegT
Degradation of a metabolite V ($V = A, F_1, F_2$)	DegV

Parameter	Symbol
Glucose	G

Table 2.1. Symbols for the variables, their production (expressed as time derivatives), parameter and primitive fluxes of the genetically regulated fatty acid metabolism.

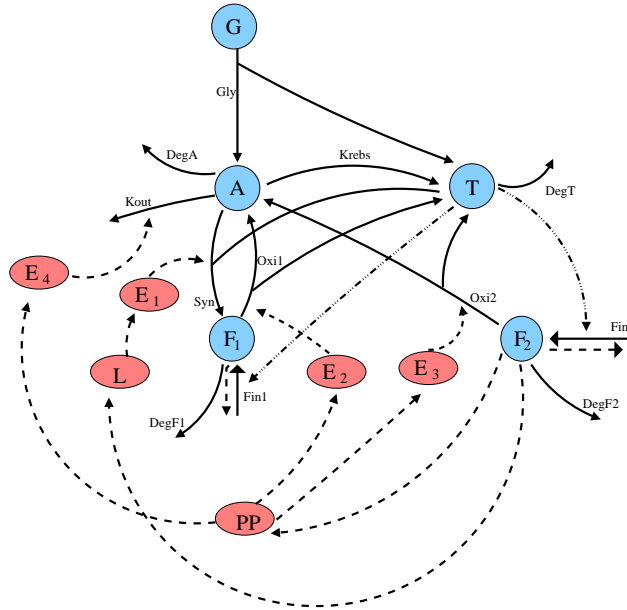*2.2. Regulations for the mixed model of fatty acid metabolism*

Fig. 2.1. A model for genetic regulations of fatty acid metabolism. Dashed arrows stand for genetic timescale actions from the origin on to target. Plain arrows stand for metabolic fluxes. Dot arrows stand for energetic regulations implying T. In this model, notice that a metabolite F_2 (that is, polyunsaturated fatty acids PUFA) regulate the genetic regulators L (LXRα-SREBP-1 pathway)) and PP (PPAR-α).

We have first defined a sketch of the model. We now intend to add the regulation relations between fluxes and variables. These are described by the sign of the variations of a flux when we increase the activity of the regulator. In mathematical terms, regulation is summarized by the set of signs of the partial

derivatives of the primitive fluxes with respect to the variables. We consider two classes of regulation, namely metabolic and genetic regulations.

Metabolic and energetic (ATP) regulations. Metabolic biochemistry has intrinsic regulation: substrates stimulate and products inhibit reactions. The latter effect is a consequence of the more general Le Chatelier principle that says briefly that effects turn against their causes. In control theory this means that feedback is negative, which is in favor of uniqueness and stability of equilibrium. Other negative feedback regulations are responses to the energetic balance (ATP/ADP or ATP/AMP ratios) via direct biochemical regulation, or more complex signalling pathways [Car05]. An increase of ATP favours catabolic processes (lipolysis and oxidation) while a decrease favours synthetic pathways.

We can classify metabolic regulations as following:

- **Substrate effect** An increase of substrate increases the associated flux. This implies the following relations in the model:

$$\frac{\partial \mathrm{Syn}}{\partial A} > 0,\ \frac{\partial \mathrm{Gly}}{\partial G} > 0,\ \frac{\partial \mathrm{Oxi1}}{\partial F_1} > 0,\ \frac{\partial \mathrm{Oxi2}}{\partial F_2} > 0,\ \frac{\partial \mathrm{Kout}}{\partial A} > 0,\ \frac{\partial \mathrm{Krebs}}{\partial A} > 0,\ \frac{\partial \mathrm{DegT}}{\partial T} > 0,\ \frac{\partial \mathrm{Syn}}{\partial T} > 0.$$

  Also, degradation reactions are modeled as $\mathrm{DegV}(V) = \delta_V V$, $\delta_V > 0$ where V denotes any variable A, F_1, F_2. Hence $\frac{\partial \mathrm{DegV}}{\partial V} > 0$.
- **Passive or active transport effects** Intake/outtake fluxes Fin1 and Fin2 are conventionally directed to the inside. Hence, they decrease when the internal concentrations of fatty acids increase: $\frac{\partial \mathrm{Fin1}}{\partial F_1} < 0$ and $\frac{\partial \mathrm{Fin2}}{\partial F_2} < 0$.
- **Product negative feed-back** Fluxes producing ATP are negatively controlled by ATP. Thus, $\frac{\partial \mathrm{Gly}}{\partial T} < 0$, $\frac{\partial \mathrm{Oxi1}}{\partial T} < 0$, $\frac{\partial \mathrm{Oxi1}}{\partial T} < 0$, $\frac{\partial \mathrm{Krebs}}{\partial T} < 0$.
- **Energy effect on fat intake** Fat intake is needed to produce energy by oxidation. A drop in energy (ATP) stimulates fat intake. This means that $\frac{\partial \mathrm{Fin1}}{\partial T} < 0$ and $\frac{\partial \mathrm{Fin2}}{\partial T} < 0$.

Genetic regulations. The role of PUFA in genetic control has been discussed in recent publications [PLM03, DF04, Jum04]. Although the precise mechanisms have not been proven yet, some well established facts can be used for modeling:

- **Fatty Acid synthesis down-regulation**. PUFA inhibit lipogenesis via the LXRα-SREBP-1 regulation path. Concerning the mechanisms of these interactions there are some hypothesis. PUFA could regulate the nuclear abundance of transcription factors such as SREBP-1 via the turnover of its mRNA and also via its proteolytic processing which is specific of SREBP's family [Jum04]. It has also been suggested that PUFA can bind to and modify the activity of nuclear receptors PPAR and LXR: active PPAR and LXR are heterodimers with RXR (retinoid X receptor): PUFA could prevent nuclear receptor LXR from forming a heterodimer with RXR, and therefore blocks its activity as a transcription factor [Jum04]..
- **Oxidation up-regulation**. PUFA stimulate their oxidation as well as the oxidation of F_1 since they activate PPAR. The detailed mechanism is not known: it either cooperative stimulation of transcriptional effect of PPAR or indirect (active PPAR is a heterodimer with RXR; preventing LXR/RXR formation fatty acids shifts the equilibrium toward PPAR/RXR formation).
- **Ketone exit up-regulation**. The mitochondrial HMG-CoA synthase, a key enzyme of the ketone body formation is known to be transactivated by PPARα; in vivo PPARα activation leads to an increase of ketone bodies exit [La04].

We translate this biological information into several relations between the variables. Notice that since these regulations imply genetic interactions, they occur only on long (genetic) timescale τ_G.

- PUFA (F_2) activates PPAR (PP) and inhibits active-LXR and SREBP-1 (L): $\frac{\partial \Psi_1}{\partial F_2} > 0$, $\frac{\partial \Psi_2}{\partial F_2} < 0$.
- LXR and SREBP-1 (L) triggers E_1 production (where E_1 models S/MU-FA synthesis enzymes): $\frac{\partial \Psi_3}{\partial L} > 0$.
- PPAR (PP) triggers the production of E_2 (S/MU-FA oxidation enzymes), E_3 (PUFA oxydation enzymes) and E_4 (ketone exit enzymes): $\frac{\partial \Psi_4}{\partial PP} > 0$, $\frac{\partial \Psi_5}{\partial PP} > 0$, $\frac{\partial \Psi_6}{\partial PP} > 0$.
- Degradation effects occurs on each genetic variable.
- Abstract enzymes E_i stimulate the corresponding fluxes. $\frac{\partial \mathrm{Syn}}{\partial E_1} > 0$, $\frac{\partial \mathrm{Oxi1}}{\partial E_2} > 0$, $\frac{\partial \mathrm{Oxi2}}{\partial E_3} > 0$, $\frac{\partial \mathrm{Kout}}{\partial E_4} > 0$.

$$
\begin{cases}
\frac{dA}{dt} = Gly(G,T) + n_1 Oxi1(F_1,T,E_2) + n_2 Oxi2(F_2,T,E_3) \\
\qquad - Krebs(A,T) - Kout(A,E_4) - n_1 Syn(A,T,E_1) - \delta_A A \\
\frac{dF_1}{dt} = Syn(A,T,E_1) - Oxi1(F_1,T,E_2) + Fin1(F_1,T) - \delta_{F_1} F_1 \\
\frac{dF_2}{dt} = -Oxi2(F_2,T,E_3) + Fin2(F_2,T) - \delta_{F_2} F_2 \\
\frac{dT}{dt} = \alpha_G Gly(G,T) + \alpha_K Krebs(A,T) + \alpha_{O1} Oxi1(F_1,T,E_2) \\
\qquad + \alpha_{O2} Oxi2(F_2,T,E_3) - \alpha_S Syn(A,T,E_1) - DegT(T) \\
\frac{dPP}{dt} = \widetilde{\Psi}_1(F_2) - \delta_{PP} PP \\
\frac{dL}{dt} = \widetilde{\Psi}_2(F_2) - \delta_L L \\
\frac{dE_1}{dt} = \widetilde{\Psi}_3(L) - \delta_{E_1} E_1 \\
\frac{dE_2}{dt} = \widetilde{\Psi}_4(PP) - \delta_{E_2} E_2 \\
\frac{dE_3}{dt} = \widetilde{\Psi}_5(PP) - \delta_{E_3} E_3 \\
\frac{dE_4}{dt} = \widetilde{\Psi}_6(PP) - \delta_{E_4} E_4
\end{cases}
$$

$\frac{\partial \text{flux}}{\partial \text{var.}}$	Gly	Krebs	Kout	Syn	Oxi1	Oxi2	Fin1	Fin2	DegT	$\widetilde{\Psi}_1$	$\widetilde{\Psi}_2$	$\widetilde{\Psi}_3$	$\widetilde{\Psi}_4$	$\widetilde{\Psi}_5$	$\widetilde{\Psi}_6$
A	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0
F_1	0	0	0	0	+	0	−	0	0	0	0	0	0	0	0
F_2	0	0	0	0	0	+	0	−	0	+	−	0	0	0	0
T	−	−	0	+	−	−	−	−	+	0	0	0	0	0	0
PP	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+
L	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
E_1	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
E_2	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0
E_3	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0
E_4	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0
G	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.2. Differential model for the regulated fatty acid metabolism: equations and constraints. The flux of each metabolic variable is obtained as a mass balance of primitive fluxes. The dependance of primitive metabolic fluxes and of genetic production terms on variables is given in the table above.

2.3. Differential model for the regulated fatty acid metabolism

The graphical representation of the model is shown in Fig.2.1. Table 2.2 summarizes a differential model including the above described relations among metabolic and genetic variables. Let us summarize the notations:

- $\mathbf{X} = (A, F_1, F_2, T)$ is the set of metabolic variables;
- $\mathbf{Y} = (PP, L, E_1, E_2, E_3, E_4)$ is the set of genetic variables;
- $\mathbf{p} = G$ is the parameter of the model; we suppose that it takes values inside a compact interval, $G \in [0, G_{max}]$.
- $\Phi : \mathbb{R}_+^{10} \to \mathbb{R}^4$ and $\Psi : \mathbb{R}_+^{10} \to \mathbb{R}^6$ are defined such that:

$$
\begin{cases}
\frac{d\mathbf{X}}{dt} = \Phi(\mathbf{X}, \mathbf{Y}, \mathbf{p}) \\
\frac{d\mathbf{Y}}{dt} = \Psi(\mathbf{X}, \mathbf{Y}, \mathbf{p})
\end{cases} .
$$

The differential model was built as follows:

- The production Φ_A, Φ_{F_1}, Φ_{F_2}, Φ_T of each metabolic variable is obtained as the sum of primitive fluxes that produce or consume the metabolite.
- Primitive fluxes are treated as single reactions with simple stoechiometry. Thus, the fluxes Gly, Krebs, Oxi1, Oxi2, Syn are considered to have the stoechiometries $G \to A + \alpha_G T$, $A \to \alpha_K T$, $F_1 \to n_1 A + \alpha_{O1} T$, $F_2 \to n_1 A + \alpha_{O2} T$, $n_1 A + \alpha_S T \to F_1$ respectively.
- Degradation reactions of metabolites are supposed to be linear: $DegV(V) = \delta_V V$ where V denotes any variable A, F_1, F_2.
- The functions Ψ_i expressing variations of the genetic variables (PP, L, E_1, E_2, E_3, E_4) were not detailed because mechanisms are still unknown. Instead, each function Ψ_i has been decomposed into a non-negative production term $\widetilde{\Psi}_i$ and a linear degradation term. Qualitative information on the production term is translated into a set of signs of its partial derivatives as in Table 2.2; for the time being these are the only a priori constraints to the model.

2.4. Reduced models for the metabolic variables

Let us construct the two reduced models and state equations for the metabolic variables: first, the genetic partial equilibrium model and its associated state equations; second, the genetically non-regulated model and its state equations.

In order to obtain the genetic partial equilibrium model we must eliminate the genetic variables from their equilibrium equations, i.e. we must solve the subsystem (genetic partial equilibrium):

$$
\begin{cases}
\widetilde{\Psi}_1(F_2) - \delta_{PP} PP = 0 , & \widetilde{\Psi}_4(PP) - \delta_{E_2} E_2 = 0 \\
\widetilde{\Psi}_2(F_2) - \delta_L L = 0 , & \widetilde{\Psi}_5(PP) - \delta_{E_3} E_3 = 0 \\
\widetilde{\Psi}_3(L) - \delta_{E_1} E_1 = 0 , & \widetilde{\Psi}_6(PP) - \delta_{E_4} E_4 = 0.
\end{cases}
\tag{2.1}
$$

$$\begin{cases} \frac{dA}{dt} = \text{Gly}(G,T) + n_1 \text{Oxi1}_{\text{peq},gnr}(F_1,F_2,T) + n_2 \text{Oxi2}_{\text{peq},gnr}(F_2,T) \\ \qquad -\text{Krebs}(A,T) - \text{Kout}_{\text{peq},gnr}(A,F_2) - n_1 \text{Syn}_{\text{peq},gnr}(A,F_2,T) - \delta_A A \\ \frac{dF_1}{dt} = \text{Syn}_{\text{peq},gnr}(A,F_2,T) - \text{Oxi1}_{\text{peq},gnr}(F_1,F_2,T) + \text{Fin1}(F_1,T) - \delta_{F_1} F_1 \\ \frac{dF_2}{dt} = -\text{Oxi2}_{\text{peq},gnr}(F_2,T) + \text{Fin2}(F_2,T) - \delta_{F_2} F_2 \\ \frac{dT}{dt} = \alpha_G \text{Gly}(G,T) + \alpha_K \text{Krebs}(A,T) + \alpha_{O1} \text{Oxi1}_{\text{peq},gnr}(F_1,F_2,T) \\ \qquad + \alpha_{O2} \text{Oxi2}_{\text{peq},gnr}(F_2,T) - \alpha_S \text{Syn}_{\text{peq},gnr}(A,F_2,T) - \text{DegT}(T) \end{cases}$$

$\frac{\partial \text{ flux}}{\partial \text{ variable}}$	Gly	Krebs	Kout	Syn	Oxi1	Oxi2	Fin1	Fin2	DegT
A	0	+	+	+	0	0	0	0	0
F_1	0	0	0	0	+	0	−	0	0
F_2 gnr	0	0	0	0	0	+	0	−	0
F_2 peq	0	0	+	−	+	+	0	−	0
T	−	−	0	+	−	−	−	−	+
G	+	0	0	0	0	0	0	0	0

a) genetically non-regulated model.

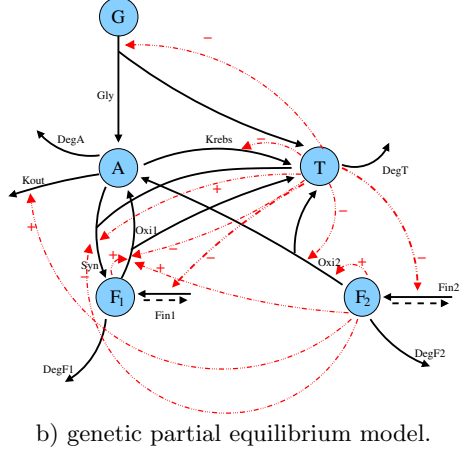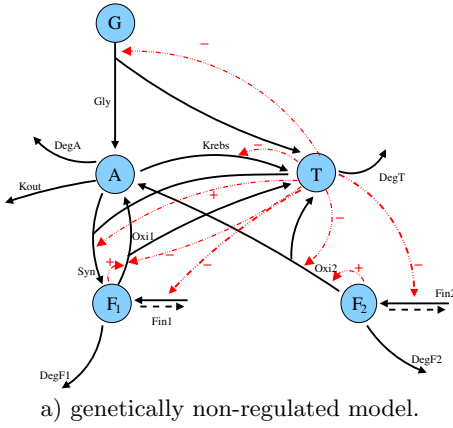b) genetic partial equilibrium model.

Table 2.3. Reduced models for the metabolic variables of the regulated fatty acid metabolism. The reduced primitive fluxes and their regulations are also represented graphically.

Notice that in the subsystem (2.1), the global variable F_2 is a parameter. If a solution of this subsystem exists, then the genetic variables $PP, L, E_1, E_2, E_3, E_4$ are expressible as functions of F_2. It can be easily shown that this is possible in a unique way, meaning that the Unique Genetic Partial Equilibrium Condition 1 is fulfilled:

Proposition 2.1. *For any nonnegative value of* F_2*, the partial equilibrium equations of genetic variables (Eq.(2.1)) admit a unique solution, i.e. the Condition 1 is fulfilled.*

Proof. Solving the partial equilibrium equations gives readily the values of genetic variables: $PP_{\text{peq}}(F_2) = \frac{1}{\delta_{PP}} \widetilde{\psi}_1(F_2)$, $L_{\text{peq}}(F_2) = \frac{1}{\delta_L} \widetilde{\psi}_2(F_2)$, $E_{1\text{peq}}(F_2) = \frac{1}{\delta_{E_1}} \widetilde{\psi}_3(L_{\text{peq}}(F_2))$, $E_{2\text{peq}}(F_2) = \frac{1}{\delta_{E_2}} \widetilde{\psi}_4(PP_{\text{peq}}(F_2))$, $E_{3\text{peq}}(F_2) = \frac{1}{\delta_{E_3}} \widetilde{\psi}_5(PP_{\text{peq}}(F_2))$ and $E_{4\text{peq}}(F_2) = \frac{1}{\delta_{E_4}} \widetilde{\psi}_6(PP_{\text{peq}}(F_2))$. ∎

Proposition 2.2. *At genetic partial equilibrium, the derivatives of the values of the genetic variables with respect to* F_2 *satisfy*

$$\frac{dPP_{\text{peq}}}{dF_2} > 0,\ \frac{dL_{\text{peq}}}{dF_2} < 0,\ \frac{dE_{1\text{peq}}}{dF_2} < 0,\ \frac{dE_{2\text{peq}}}{dF_2} > 0,\ \frac{dE_{3\text{peq}}}{dF_2} > 0,\ \frac{dE_{4\text{peq}}}{dF_2} > 0.$$

Proof. We use the chain rule formula. For example: $\frac{dPP_{\text{peq}}}{dF_2} = \frac{1}{\delta_{PP}} \frac{\partial \widetilde{\psi}_1}{\partial F_2} > 0$. ∎

The next step of the reduction is to express the primitive fluxes at partial equilibrium as functions of metabolic variables only. For instance, $\text{Oxi2}_{\text{peq}}(F_2,T) = \text{Oxi2}(F_2,T,E_{3\text{peq}}(F_2))$.

Proposition 2.3. *At genetic partial equilibrium, the fluxes* Syn*,* Oxi1*,* Oxi2 *and* Kout *become functions of* A, F_1, F_2, T *only, denoted by* Syn_{peq}*,* Oxi1_{peq}*,* Oxi2_{peq}*,* Kout_{peq}*. The dependence of these functions on* F_2 *satisfy:*

$$\frac{\partial \text{Syn}_{\text{peq}}}{\partial F_2} < 0, \qquad \frac{\partial \text{Oxi1}_{\text{peq}}}{\partial F_2} > 0, \qquad \frac{\partial \text{Oxi2}_{\text{peq}}}{\partial F_2} > 0, \qquad \frac{\partial \text{Kout}_{\text{peq}}}{\partial F_2} > 0.$$

Proof. By definition $\text{Syn}_{\text{peq}}(A,T) = \text{Syn}(A,T,E_{1\text{peq}}(F_2))$. Then $\frac{\partial \text{Syn}_{\text{peq}}}{\partial F_2} = \frac{\partial \text{Syn}}{\partial E_1} \frac{dE_{1\text{peq}}}{dF_2}$. By Lemma 2.2 and Table 2.1 it follows that $\frac{\partial \text{Syn}_{\text{peq}}}{\partial F_2} < 0$.

The sign of the other derivatives is computed in a similar way. ∎

In genetically non-regulated states genetic variables are constant, equal to their initial values:

$$\text{E}_1(t) = \text{E}_1(0), \text{E}_2(t) = \text{E}_2(0), \text{E}_3(t) = \text{E}_3(0), \text{E}_4(t) = \text{E}_4(0) \qquad (2.2)$$

Primitive fluxes are functions of metabolic variables only and their derivatives follow directly from the Table 2.1:

Proposition 2.4. *In genetically non-regulated states, the fluxes* Syn*, *Oxi1*, *Oxi2 *and* Kout *become functions of* $\text{A}, \text{F}_1, \text{F}_2, \text{T}$ *only, denoted by* Syn_{gnr}, Oxi1_{gnr}, Oxi2_{gnr}, Kout_{gnr}. *The dependence of these functions on* F_2 *is as following:*

$$\frac{\partial \text{Syn}_{gnr}}{\partial \text{F}_2} = 0, \qquad \frac{\partial \text{Oxi1}_{gnr}}{\partial \text{F}_2} = 0, \qquad \frac{\partial \text{Oxi2}_{gnr}}{\partial \text{F}_2} > 0, \qquad \frac{\partial \text{Kout}_{gnr}}{\partial \text{F}_2} = 0$$

Table 2.3 summarizes the reduced models in the two situations together with the table of constraints (signs of the partial derivatives of the reduced primitive fluxes with respect to the metabolic variables).

3. Equilibrium and quasi-stationary states: existence and uniqueness

The process of elimination of variables simplifies the study of equilibrium states of the model. We have eliminated the genetic variables in order to obtain the state equations for metabolic variables. These state equations have to be solved. The solutions of state equations are equilibria or quasi-stationary states. In this section we focus on the number of solutions. As discussed in the introduction if equilibria are unique, modifications of the variables induced by (slow) changes of the parameter of the model (food) are smooth equilibrium shifts. The same is true for quick changes if quasi-stationary states are unique. In order to find solutions of the state equations we proceed by further elimination of variables. The technical details are given in Section 4.

3.1. Existence of an equilibrium and of an quasi-stationary state

The concentration of metabolites results from the balance of production fluxes and degradation or consumption fluxes.

Until now, we have assumed the following conditions on the elementary fluxes:

Condition 2 (Flux global constraints)
– *The fluxes are differentiable functions of the concentrations and satisfy differential constraints (signs of partial derivatives), summarized in Table 2.2.*
– *Degradation terms are linear:* $\text{DegV}(\text{V}) = \delta_{\text{V}}\text{V}$ *where* V *denotes any variable* P, A, F_1, F_2.

These hypotheses are very mild. In order to go on with the analysis, we now add some more assumptions which are natural and not restrictive:

Condition 3 (Boundary and asymptotic conditions)
– *In the absence of substrates all fluxes vanish.*
– *All fluxes except degradation saturate at high concentrations of metabolites.*
– *ATP consumption is an increasing function of ATP with no saturation effect, that is* $\lim_{T\to\infty} \text{DegT} = +\infty$. *This is consistent with the fact that cells can not store ATP.*
– *There exists a recovery effect on each metabolic variable. By recovery effect we mean that if a variable is zero, then at least one elementary flux that produces the variable is activated. In particular, if the cell contains no PUFA, then PUFA enter the cell.*

By a mathematical argument, we can prove that under these assumptions, our model has at least a quasi-stationary state and at least an equilibrium. Details are given in Section 6. Let us suppose that the glucose concentration can change between 0 and a maximal value G_{max}.

Theorem 3.1 (Existence of equilibrium). *Let us suppose that the conditions 2 and 3 are satisfied for all glucose concentrations within an interval* $0 \leq \text{G} \leq G_{max}$. *Then, the genetically non-regulated model (Eq.(2.2) and Table 2.3) and the genetic partial equilibrium model (Eq.(2.1) and Table 2.3) for fatty acid metabolism admit at least an equilibrium state for every* $0 \leq \text{G} \leq G_{max}$.

According to Proposition 1.1, we derive a result about the full model.

Biological prediction 1
Under the conditions of Theorem 3.1 the regulated fatty acids metabolism model described in Table 2.2 has at least a quasi-stationary state and at least an equilibrium.

Various methods using the *interaction graph* provide sufficient criteria for the uniqueness of equilibrium of a differential model [Tho81, Sno98, Gou98, Sou03]. In this section, we show that those methods do not apply to our case and we propose a different method.

Interaction graphs for metabolic variables. Oriented interaction graphs can be defined for systems of differential equations [Sou03]. The interaction graph gathers information relative to the (direct or indirect) action of a variable on another one and is thus important in the theory of response [RLS$^+$06]. The interaction graph can be computed at genetic partial equilibrium or at fixed genetic variables (in genetically non-regulated states) as follows. There is an arc from X_i to X_j whenever $\frac{\partial \Phi_j^{red}}{\partial X_i} \neq 0$ meaning that X_i has an influence on the flux of X_j. The sign of the regulation arc is the sign of the derivative $\frac{\partial \Phi_j^{red}}{\partial X_i}$. At fixed genetic variables the interaction graph gathers purely metabolic influences between metabolites; it corresponds to response on timescales that are too short to allow for genetic readjustments. At genetic partial equilibrium the interaction graph gathers both metabolic and genetically mediated influences between metabolites.

In order to build the interaction graphs, we need to compute the signs of the derivatives of the fluxes with respect to the metabolites. This is done in the Table 3.1 by using Table 2.3. The sign of the fluxes of metabolites is computed like in the following example (corresponding to genetic partial equilibrium):

$$\frac{\partial \Phi_{F_1}^{peq}}{\partial F_1} = \frac{\partial}{\partial F_1}[\mathrm{Syn}_{peq}(A, F_2, T) - \mathrm{Oxi1}_{peq}(F_1, F_2, T) + \mathrm{Fin1}(F_1, T) - \delta_{F_1} F_1] =$$
$$= -\underbrace{\frac{\partial}{\partial F_1}\mathrm{Oxi1}_{peq}(F_1, F_2, T)}_{>0} + \underbrace{\frac{\partial}{\partial F_1}\mathrm{Fin1}(F_1, T)}_{<0} - \underbrace{\delta_{F_1}}_{>0} < 0.$$

$\frac{\partial\,\text{flux}}{\partial\,\text{variable}}$	Φ_A	Φ_{F_1}	Φ_{F_2}	Φ_T
A	$-$	$+$	0	(I)
F_1	$+$	$-$	0	$+$
F_2 gnr / peq	$+$ / (II)	0 / $-$	$-$	$+$
T	(III)	(IV)	(V)	$-$
G	$+$	0	0	$+$

a) non-genetically regulated model (fixed genetic variables, metabolic influences)

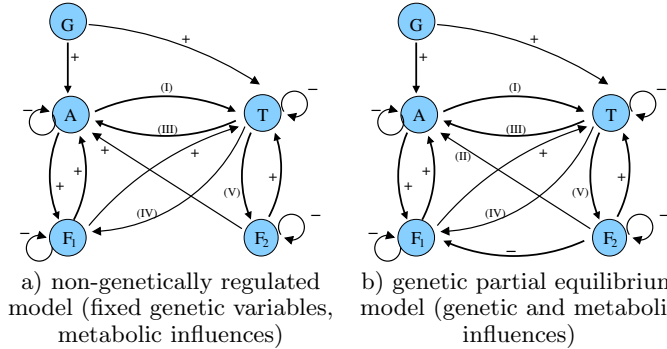b) genetic partial equilibrium model (genetic and metabolic influences)

Table 3.1. Signs of the partial derivatives of the fluxes and interaction graphs for the models derived from the regulated fatty acid metabolism model at fixed genetic variables and genetic partial equilibrium. The roman numerals indicate partial derivatives and regulation arcs whose signs depend on the point where they are computed.

Simple topological conditions for the uniqueness of equilibrium do not apply to any of these interaction graphs. Thomas rule asserts that the absence of positive loops in the interaction graph for all values of the node variables and external parameters is a sufficient condition for the uniqueness of equilibrium [Tho81, Sno98, Gou98, Sou03]. Unfortunately, it appears that Thomas rule can not be applied in our case as stated in the following proposition.

Proposition 3.1. *The interaction graphs of the genetically non-regulated and of the genetic partial equilibrium models for the regulated fatty acid metabolism have both at least a positive loop for any value of the variables and of the external parameter* G.

Proof. There exists a positive loop between A and F_1 (see Table 3.1). ∎

In the following, we develop another method to prove the uniqueness of equilibrium. This method proceeds by successive eliminations of variables until we are left with only one equation. Then we write

down a sufficient condition for having a unique solution to that last equation. The success of the method depends on the appropriate gathering of variables that can be simultaneously eliminated in an unique way. This is described in full generality in Section 6.

Uniqueness of equilibrium and of the quasi-stationary state A maximal set of variables that can be eliminated in a unique way from the state equations is $\{A, F_1, F_2\}$. In order to eliminate them, we consider the following state equations, at fixed T and G (those are both considered as parameters here):

$$\Phi_A^{red}(G, A, F_1, F_2, T) = 0, \ \Phi_{F_1}^{red}(A, F_1, F_2, T) = 0, \ \Phi_{F_2}^{red}(F_2, T) = 0. \tag{3.1}$$

Let us recall that Φ^{red} is either Φ_{gnr} or Φ_{peq} depending on the situation: at fixed genetic variables or at genetic partial equilibrium.

Proposition 3.2. *Suppose that the Conditions 2 and 3 are fulfilled for $0 \leq G \leq G_{max}$ and $T \geq 0$. Then the system (3.1) admits a unique solution for any given pair of values $(G, T) \in [0, G_{max}] \times \mathbb{R}_+$, both at fixed genetic variables and at genetic partial equilibrium.*
  The functions $A^{(1)}(G, T)$, $F_1^{(1)}(G, T)$, $F_2^{(1)}(G, T)$ expressing this solution are differentiable in (G, T).

To ensure uniqueness of equilibrium, we need to eliminate T from the state equation

$$\Phi_T^{(1)}(T, G) = \Phi_T^{red}(G, A^{(1)}(G, T), F_1^{(1)}(G, T), F_2^{(1)}(G, T), T) = 0.$$

A sufficient condition for the unique elimination of T is $\frac{d\Phi_T^{(1)}}{dT} < 0$. In Section 6 we show that $\frac{d\Phi_T^{(1)}}{dT}$ is a linear combination with positive coefficients of derivatives with respect to T; this allows us to restate the uniqueness conditions as follows:

Condition 4 (Strong lipolytic condition)
The following inequality is satisfied for any $(G, T) \in [0, G_{max}] \times \mathbb{R}_+$:
$$\frac{\partial A^{(1)}}{\partial T}\left(\alpha_K \frac{\partial Krebs}{\partial A} - \frac{\alpha_K}{n_1}\frac{\partial Syn}{\partial A}\right) + \frac{\partial F_1^{(1)}}{\partial T}\alpha_{O1}\frac{\partial Oxi1}{\partial F_1} + \frac{\partial F_2^{(1)}}{\partial T}\left(\alpha_{O1}\frac{\partial Oxi1}{\partial F_2} - \alpha_S\frac{\partial Syn}{\partial F_2} + \alpha_{O2}\frac{\partial Oxi2}{\partial F_2}\right) + \frac{\partial \Phi_T}{\partial T} < 0.$$

Theorem 3.2 (Uniqueness of equilibrium). *Suppose that the Conditions 2, 3, 4 are fulfilled at fixed genetic variables and at genetic partial equilibrium, for every $G \in [0, G_{max}]$.*
  Then, the state equations (3.1) for metabolic variables admit a unique solution for every $G \in [0, G_{max}]$, both at fixed genetic variables and at genetic partial equilibrium. The concentration of metabolites and ATP at equilibrium are differentiable functions of G: $A^{(2)}(G)$, $F_1^{(2)}(G)$, $F_2^{(2)}(G)$, $T^{(2)}(G)$.
  In other words, the equilibrium and the quasi-stationary states of the full model for genetic regulations of fatty acid metabolism are unique.

Biological prediction 2 (Shift mechanism)
When the Conditions 1,2,3,4 are satisfied, the mechanism allowing the change of functioning modes (lipogenesis and oxidation) is a shift, both for rapid response (quasi-stationarity) and for slow static response (equilibrium).

3.3. Computable version and biological significance of the strong lipolytic condition

In practice, the strong lipolytic condition 4 is not readily exploitable because it contains the derivatives of implicit functions $A^{(1)}$, $F_1^{(1)}$, $F_2^{(1)}$. In order to give a computable version of it, we express the above derivatives by using control coefficients and metabolic elasticities.

Control coefficients, Metabolic elasticities. In metabolic control language, a control coefficient quantifies the dependency of a flux on an enzyme activity. The general idea of control coefficients is that they permit to compare the strength of fluxes variations one with respect to the other. Following [CB95], we call *control coefficient of a flux* the derivative of the logarithm of the flux with respect to the logarithm of the enzyme concentration. In our problem, although they are not enzymes, F_2 and T play regulatory roles on the fluxes through genetic and metabolic factors. Notice that these effects are absent in classical metabolic control analysis (this focuses on the effect of enzymes). Thus we choose to call *non-logarithmic control coefficients* all the following quantities:

– genetic (non-logarithmic) control coefficients:

$$R_{\mathrm{F_2}}^{\mathrm{Oxi2}} = \frac{\partial \mathrm{Oxi2}_{\mathrm{peq},gnr}}{\partial \mathrm{F_2}}, \quad R_{\mathrm{F_2}}^{\mathrm{Oxi1}} = \frac{\partial \mathrm{Oxi1}_{\mathrm{peq},gnr}}{\partial \mathrm{F_2}}, \quad R_{\mathrm{F_2}}^{\mathrm{Syn}} = -\frac{\partial \mathrm{Syn}_{\mathrm{peq},gnr}}{\partial \mathrm{F_2}}, \quad R_{\mathrm{F_2}}^{\mathrm{Kout}} = \frac{\partial \mathrm{Kout}_{\mathrm{peq},gnr}}{\partial \mathrm{F_2}}.$$

– ATP (non-logarithmic) control coefficients:

$$R_{\mathrm{T}}^{\mathrm{Fin1}} = -\frac{\partial \mathrm{Fin1}}{\partial \mathrm{T}}, \quad R_{\mathrm{T}}^{\mathrm{Fin2}} = -\frac{\partial \mathrm{Fin2}}{\partial \mathrm{T}}, \quad R_{\mathrm{T}}^{\mathrm{Syn}} = \frac{\partial \mathrm{Syn}_{\mathrm{peq},gnr}}{\partial \mathrm{T}}, \quad R_{\mathrm{T}}^{\mathrm{Oxi1}} = -\frac{\partial \mathrm{Oxi1}_{\mathrm{peq},gnr}}{\partial \mathrm{T}}.$$

$$R_{\mathrm{T}}^{\mathrm{Oxi2}} = -\frac{\partial \mathrm{Oxi2}_{\mathrm{peq},gnr}}{\partial \mathrm{T}}, \quad R_{\mathrm{T}}^{\mathrm{Krebs}} = -\frac{\partial \mathrm{Krebs}}{\partial \mathrm{T}}, \quad R_{\mathrm{T}}^{\mathrm{Gly}} = -\frac{\partial \mathrm{Gly}}{\partial \mathrm{T}}.$$

These quantities were defined such that they are all positive (see Proposition 3.3). This sign choice simplifies the identification of balances in the interaction graph. For instance the interactions in the interaction graphs with undetermined signs can be expressed as sums and differences of positive control coefficients:

(II) $\frac{\partial \Phi_{\mathrm{A}}}{\partial \mathrm{F_2}} = n_1 R_{\mathrm{F_2}}^{\mathrm{Oxi1}} + n_1 R_{\mathrm{F_2}}^{\mathrm{Syn}} + n_2 R_{\mathrm{F_2}}^{\mathrm{Oxi2}} - R_{\mathrm{F_2}}^{\mathrm{Kout}},$ $\qquad (IV)$ $\frac{\partial \Phi_{\mathrm{F_1}}}{\partial \mathrm{T}} = R_{\mathrm{T}}^{\mathrm{Syn}} + R_{\mathrm{T}}^{\mathrm{Oxi1}} - R_{\mathrm{T}}^{\mathrm{Fin1}},$

(III) $\frac{\partial \Phi_{\mathrm{A}}}{\partial \mathrm{T}} = R_{\mathrm{T}}^{\mathrm{Krebs}} - R_{\mathrm{T}}^{\mathrm{Gly}} - n_1 R_{\mathrm{T}}^{\mathrm{Syn}} - n_1 R_{\mathrm{T}}^{\mathrm{Oxi1}} - n_2 R_{\mathrm{T}}^{\mathrm{Oxi2}},$ $\qquad (V)$ $\frac{\partial \Phi_{\mathrm{F_2}}}{\partial \mathrm{T}} = R_{\mathrm{T}}^{\mathrm{Oxi2}} - R_{\mathrm{T}}^{\mathrm{Fin2}}.$

The non-logarithmic control coefficients can be defined for all values of the metabolic variables. In particular, at fixed genetic variables the relations $(R_{\mathrm{F_2}}^{\mathrm{Oxi1}})_{gnr} = 0$, $(R_{\mathrm{F_2}}^{\mathrm{Syn}})_{gnr} = 0$, $(R_{\mathrm{F_2}}^{\mathrm{Kout}})_{gnr} = 0$ express the absence of genetic regulation. The coefficient $R_{\mathrm{F_2}}^{\mathrm{Oxi2}}$ is the sum of a positive metabolic and a positive genetic contribution. The genetic contribution vanishes at fixed genetic variables, hence $0 < (R_{\mathrm{F_2}}^{\mathrm{Oxi1}})_{gnr} < (R_{\mathrm{F_2}}^{\mathrm{Oxi1}})_{peq}$.

Following [CB95], we call *elasticity* the derivative of the logarithm of the rate with respect to the logarithm of the substrate concentration. In our setting we call *non-logarithmic elasticities* the following coefficients: they quantify how rates and fluxes of a metabolite depend on this metabolite. Recall that the rates Φ_V are sums of primitive fluxes in Table 2.3.

$$\chi_{\mathrm{F_1}}^{tot} = -\frac{\partial \Phi_{\mathrm{F_1}}}{\partial \mathrm{F_1}}, \quad \chi_{\mathrm{F_2}}^{tot} = -\frac{\partial \Phi_{\mathrm{F_2}}}{\partial \mathrm{F_2}}, \quad \chi_{\mathrm{A}}^{tot} = -\frac{\partial \Phi_{\mathrm{A}}}{\partial \mathrm{A}}, \quad \chi_{\mathrm{A}}^{\mathrm{Syn}} = \frac{\partial \mathrm{Syn}_{\mathrm{peq}}}{\partial \mathrm{A}}, \quad \chi_{\mathrm{A}}^{\mathrm{Krebs}} = \frac{\partial \mathrm{Krebs}}{\partial \mathrm{A}}, \quad \chi_{\mathrm{F_1}}^{\mathrm{Oxi1}} = \frac{\partial \mathrm{Oxi1}_{\mathrm{peq}}}{\partial \mathrm{F_1}}.$$

Then the last interaction with undetermined sign in the interaction graph can be expressed as (I) : $\frac{\partial \Phi_{\mathrm{T}}}{\partial \mathrm{A}} = \alpha_{\mathrm{K}} \chi_{\mathrm{A}}^{\mathrm{Krebs}} - \alpha_{\mathrm{S}} \chi_{\mathrm{A}}^{\mathrm{Syn}}$.

It will be useful in the following to introduce the following *elasticity ratios* which evaluate the contribution of one primitive flux to the total elasticity of a metabolite.

$$\rho_{\mathrm{F_1}}^{\mathrm{Oxi1}} = \frac{\chi_{\mathrm{F_1}}^{\mathrm{Oxi1}}}{\chi_{\mathrm{F_1}}^{tot}}, \qquad \rho_{\mathrm{A}}^{\mathrm{Syn}} = \frac{n_1 \chi_{\mathrm{A}}^{\mathrm{Syn}}}{\chi_{\mathrm{A}}^{tot}}.$$

Also it will be useful to consider the genetic control coefficient $R_{\mathrm{F_2}}^{\mathrm{Oxi2}}$ as an elasticity and to define its associated elasticity ratio:

$$\rho_{\mathrm{F_2}}^{\mathrm{Oxi2}} = \frac{R_{\mathrm{F_2}}^{\mathrm{Oxi2}}}{\chi_{\mathrm{F_2}}^{tot}}.$$

The values of the above defined quantities must satisfy the following:

Proposition 3.3. *The genetic control coefficients, the ATP control coefficients and the elasticities are non negative. The elasticity ratios are well defined, non negative and less than 1.*

Proof. From Table 2.3, we can easily prove that genetic control coefficients and ATP control coefficients are all positive. At fixed genetic variables , some of the coefficients vanish ($R_{\mathrm{F_2}}^{\mathrm{Oxi1}} = R_{\mathrm{F_2}}^{\mathrm{Syn}} = R_{\mathrm{F_2}}^{\mathrm{Kout}} = 0$). From this analysis and Table 3.1, we get that all elasticities and the implicit ATP control coefficient are positive and the elasticity ratio are well defined, non negative and smaller than 1. ∎

Let us now restate the results of the previous sub-section in terms of control coefficients and elasticities. First, let us suppose that the stoechiometry condition is fulfilled:

Condition 5 (Stoechiometric condition)
The stoechiometric coefficients satisfy the inequalities: $\alpha_S < \alpha_{O1} < n_1\alpha_G$, $n_2\alpha_{O1} < n_1\alpha_{O2}$.

The stoechiometric condition can be checked from biochemical data. Indeed n_1, n_2 are the numbers of Acetyl-coA and α_{O1}, α_{O2} are the numbers of ATP molecules produced (in the average by different fatty acids of the same type) by oxidation of a molecule of S/MU-FA, or PUFA respectively. α_S is the average number of ATP necessary for the synthesis of a molecule of S/MU-FA. β-oxidation produces 5 molecules of ATP for each released molecule of Acetyl-coA, thus $\alpha_{O1} = 5(n_1 - 1)$, $\alpha_{O2} = 5(n_2 - 1)$. PUFA have in the average longer chains than de novo synthesized fatty acids, meaning that $n_2 > n_1$. We deduce $n_2\alpha_{O1} < n_1\alpha_{O2}$. Synthesis consumes less ATP than oxidation (for example, for palmitic acid $\alpha_S = 23$, $\alpha_{O1} = 35$); by generalization, we get $\alpha_S < \alpha_{O1}$. Finally, $\alpha_G = 7$, $\alpha_K = 12$ (these represent the number of ATP molecules produced by glycolysis and Krebs cycle per each molecule of Acetyl-CoA), from which $\alpha_{O1} < n_1\alpha_G$.

Let us define the following combinations of control coefficients:

$$
\begin{aligned}
&A = X\rho_{F_1}^{\text{Oxi1}}, \qquad X = n_1(\alpha_{O1} - \alpha_S\rho_A^{\text{Syn}} + n_1\alpha_K\rho_A^{Krebs}), \\
&B = B_1 R_{F_2}^{\text{Syn}}/\chi_{F_2}^{tot} + B_2 R_{F_2}^{\text{Kout}}/\chi_{F_2}^{tot} + B_3 R_{F_2}^{\text{O1}}/\chi_{F_2}^{tot} + B_4\rho_{F_2}^{\text{Oxi2}}, \\
&B_1 = X - n_1(\alpha_{O1} - \alpha_S)(1 - \rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}}), \qquad B_2 = \alpha_{O1}(1 - \rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}}) - X/n_1, \\
&B_3 = X(1 - \rho_{F_1}^{\text{Oxi1}}), \qquad B_4 = n_1\alpha_{O2}(1 - \rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}}) + n_2/n_1 X - n_2\alpha_{O1}(1 - (\rho_A^{\text{Syn}})^2\rho_{F_1}^{\text{Oxi1}}), \\
&C = [X/n_1 + (n_1\alpha_G - \alpha_{O1})(1 - \rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}})R_T^{Gly} + [\alpha_{O1} + n_1\alpha_K + XR_T^{Oxi1} \\
&\qquad [n_2/n_1 X + n_2\alpha_S\rho_A^{\text{Syn}}(1 - \rho_{F_1}^{\text{Oxi1}}) + (n_1\alpha_{O2} - n_2\alpha_{O1})(1 - \rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}})]R_T^{Oxi2} + \\
&\qquad -(\alpha_S + X/n_1)\rho_{F_1}^{\text{Oxi1}} + \rho_A^{\text{Syn}}]R_T^{Krebs} + [n_1(\alpha_S - \alpha_{O1}) + X](1 - \rho_{F_1}^{\text{Oxi1}})R_T^{Syn}, \\
&D = [X/n_1 - \alpha_{O1}(1 - \rho_A^{\text{Syn}})]R_T^{Krebs} + n_2\alpha_S\rho_A^{\text{Syn}}(1 - \rho_{F_1}^{\text{Oxi1}})R_T^{Oxi2} + n_1(\alpha_{O1} - \alpha_S)(1 - \rho_A^{\text{Syn}})\rho_{F_1}^{\text{Oxi1}}R_T^{S}.
\end{aligned}
$$
(3.2)

The strong lipolytic condition is equivalent to the following, more explicit condition:

Proposition 3.4. *The strong lipolytic response condition reads:*

$$A(R_T^{\text{Fin1}} - R_T^{\text{Oxi1}}) + B(R_T^{\text{Fin2}} - R_T^{\text{Oxi2}}) + C > D \tag{3.3}$$

Furthermore, if the stoechiometric condition 5 is fulfilled, then the combinations of control coefficients X, A, B_1, B_4, C, D defined by Eq.3.2 are positive.

In fact, fluxes of F_2 are much smaller than fluxes of F_1. Further simplification of the condition is reasonable:

Proposition 3.5. *If $|B(R_T^{\text{Fin2}} - R_T^{\text{Oxi2}})| <<< A|R_T^{\text{Fin1}} - R_T^{\text{Oxi1}}|$, then the strong lipolytic response condition reads:*

$$A(R_T^{\text{Fin1}} - R_T^{\text{Oxi1}}) + C > D \text{ where } A, C, D > 0. \tag{3.4}$$

Comments.

– In Eqs.(3.3),(3.4) all control coefficients are functions of G and T because the strong lipolytic condition 4 has to be checked for all $(G, T) \in [0, G_{max}] \times \mathbb{R}_+$.
– The conditions in Eqs.(3.3),(3.4) may seem more complicated than the Condition 4. Nevertheless, they are readily computable and they provide the biological significance of the strong lipolytic condition. Eq.(3.4) is fulfilled if $R_T^{\text{Fin1}} - R_T^{\text{Oxi1}}$ is large enough, which means that the energy variation has a sufficiently strong effect on the arrival of fatty acids inside the cell. This justifies the name strong lipolytic response condition.

Biological prediction 3
Under the hypothesis of 3.5, the strong lipolytic condition means that the energy variation has a sufficiently strong effect on the arrival of fatty acids inside the cell.

4. Validation, prediction and illustration of the model

Our model of regulated fatty acid metabolism can be considered at different levels.

At a *qualitative level*, the model consists of a set of differential equations, together with the differential constraints in Table 2.2. The functions giving the fluxes are not specified, neither the numerical constants involved in these functions. In order to validate the model or make some predictions, we provide sufficient qualitative conditions under which the model has a certain behavior.

If the behavior has been proven experimentally, the conditions should be added to the qualitative model as extra constraints to provide a valid model. If the behavior is a hypothesis not yet proven, the satisfiability of the sufficient condition provides predictive models. For instance the strong lipolytic condition guarantees the uniqueness of equilibrium. We do not know experimentally whether the equilibrium is unique or not. This could be tested by response experiments, by looking at the absence or at the presence of (as in the case of the operon lactose of E.coli) hysteresis in the response curves.

In this section, our aim is to determine sufficient conditions either to render from the known behaviors of the system or to have predictions on this behavior. Ideally, the conditions should accept biological interpretation. The type of behaviors that we intend to discuss within this approach are about signs of variations of metabolite concentrations in fasting/refeeding protocols (rather standard in biological studies of metabolism).

Quantitative versions of our model can be obtained by replacing the undetermined functions in Table 2.2 by specific functions containing numerical constants. The advantage is that we can probe the dynamical behavior, much easier than in qualitative models. Specifying realistic functions and constants is an enormous task for such complex biological systems. In vitro measurements of the kinetical constants are rarely available and are not always reliable. Furthermore, low complexity abstractions are only very approximate models. Thus, only robust features of dynamics of the model (that are stable against changes of the parameters or of the forms of functions) are meaningful. We shall use quantitative versions of the model as illustrations of robust dynamical behaviors.

4.1. Fatty acids concentration increase at fasting

Let us suppose that fluxes in the qualitative model satisfy the Conditions 2,3,4. Then, a unique equilibrium state and a unique quasistationary state exist for any value of the glucose concentration $G \in [0, G_{max}]$.

The next result is about the dependance of PUFA concentration on glucose. In order to state our result, let us notice that at equilibrium or at quasi-stationarity the control coefficients $R_{\mathrm{T}}^{\mathrm{Fin2}}, R_{\mathrm{T}}^{\mathrm{Oxi2}}$ depend only on G.

Proposition 4.1. *Suppose that the Conditions 1-5 are satisfied. Let* $\mathrm{F}_2^{(2)}(\mathrm{G})$ *be the value of* T *at equilibrium or quasi-stationarity (function of* G*). Then the sign of* $\frac{d\mathrm{F}_2^{(2)}}{d\mathrm{G}}$ *is equal to the sign of* $R_{\mathrm{T}}^{\mathrm{Oxi2}} - R_{\mathrm{T}}^{\mathrm{Fin2}}$.

This proposition can be stated in biological terms as follows.

Biological prediction 4 (response of PUFAs during fasting)
Suppose that the Conditions 1-5 are satisfied. The following predictions are valid for rapid (at quasi-stationarity) as well as for slow (at equilibrium) response:

- *If* $\left(R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}\right)_{eq,qs} > 0$ *for any* $0 \leq \mathrm{G} \leq G_{max}$, *then PUFAs increase during fasting and decrease during feeding.*
- *If* $\left(R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}\right)_{eq,qs} < 0$ *for any* $0 \leq \mathrm{G} \leq G_{max}$, *then PUFAs decrease during fasting and increase during feeding.*

In this case, the value of $R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}$ implies two distinct behaviors. This means that the qualitative constraints associated to the differential are not precise enough to decide which behavior occurs. However, in this case, some biological information is avalaible about the behavior of PUFAs during fasting. We will use this information to refine the model with an additional qualitative constraints.

More precisely, Lee et al. [La04] studied for wild-type and PPAR-/- mutant murine liver, the fatty acids profiles in triglycerides (TG), which are the predominant (> 50%) hepatic fatty acids and also in phospholipids (PL) which go into cellular membranes. Let us recall that TG and PL are storage forms of

fatty acids and that PL contribute much less than TG to the total fatty acid mass. These authors [La04] show that for wild type hepatocytes after 72h of fasting fatty acids profiles do not change significantly in PL, but there is a strong increase of TG and of their fatty acids constituents, in particular PUFA. Based on these experimental findings, we make the hypothesis of a mass increase during fasting, of regulating PUFA in the hepatic cell, and look for sufficient conditions ensuring this behavior in our qualitative model. This is consistent with the regulation role of PUFA: in order to trigger oxidation a persistent increase of PUFA concentration is needed inside the cell. A qualitative reasoning using an extended genetically regulated model of lipogenesis [RLS$^+$06] gives further support to this hypothesis.

Consequently, we have to add $\left(R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}\right)_{eq,qs} > 0$ as a qualitative constraint to the model to fit with behaviors observed in [La04]. By this way, we make a refinement of the model.

Condition 6 (Experimental constraint added to the model)
A necessary condition for the observed behavior of PUFAs at fasting (increase) is $\left(R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}\right)_{eq,qs} > 0$*, meaning that at equilibrium (or quasi-stationarity) the intake control overcomes the oxidation control for PUFA.*

Remark The strong lipolytic response condition, which is sufficient for the uniqueness of equilibrium asks that the intake control overcomes the oxidation control for S/MU-FA fatty acids (the major contribution to the total mass of fatty acids). Fasting experiments imply that the strong lipolytic is satisfied for all fatty acids, including PUFA. Nevertheless, this is not an experimental proof for the uniqueness of equilibrium, because one needs the strong lipolytic condition to be satisfied, not only at equilibrium (or quasi-stationarity), but in all T constrained states as well. Unfortunately, these states are not accessible experimentally.

The next result is a dynamical one. This may seem paradoxical, because in this paper we study equilibria and equilibria shifts which is a statical problem. Nevertheless, the definition of quasi-stationarity is based on dynamical timescales. Since quasi-stationarity is reached before equilibrium, comparing these two states provides some information on the dynamics of the mixed differential model.

In order to formulate the next result let us denote by B_{eq}, B_{qs} the values at equilibrium and at quasi-stationarity of the combination of control coefficients B by Eq.(3.2).

Proposition 4.2. *If the Conditions 1-6 are satisfied and if furthermore* $B_{eq} > B_{qs}$ *then* $\left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{qs} > \left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq}$*.*

This result means that when G decreases (fasting), the value of F_2 at quasi-stationarity is greater than the value at equilibrium. Thus, we can state the following prediction of the model.

Biological prediction 5 (Overshoot of fatty acid concentration)
Under the hypothesis of Prop. 4.2, the curves representing PUFA concentration during fasting must show an overshoot: the increase in concentration is greater immediately at quasi-stationarity than later at equilibrium.

Comments.

– The condition $B_{eq} > B_{qs}$ is equivalent to $B_1 \left(R_{\mathrm{F}_2}^{\mathrm{Oxi1}}\right)_{eq} + B_2 \left(R_{\mathrm{F}_2}^{\mathrm{Kout}}\right)_{eq} + B_3 \left(R_{\mathrm{F}_2}^{\mathrm{Syn}}\right)_{eq} + B_4 \frac{\partial \mathrm{Oxi2}}{\partial E3} \frac{\partial E3}{\partial \mathrm{F}_2} > 0$, with $B_1 > 0$, $B_4 \frac{\partial \mathrm{Oxi2}}{\partial E3} \frac{\partial E3}{\partial \mathrm{F}_2} > 0$. This means that even if B_2, B_3 are negative the oxidation control term is strong enough to win. At fasting, this is a plausible supposition.
– The existence of an overshoot of faty acids concentration during fasting depends on the dynamical accesibility of the quasi-stationary state. This state is accesible for a discontinous step-like glucose input, but may not be accesible if the glucose input drops slowly (see also the sections 4.4, 6.1).

4.2. Genetic regulation reinforces energy homeostasis

With the same methods, we prove in Section 6 how the values at equilibrium respond to the variations of the entering node G: not surprisingly, we predict that ATP decreases at fasting.

Proposition 4.3. *If the Conditions 1-5 are satisfied, then $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}} > 0$ at at equilibrium or quasi-stationarity, where $\mathrm{T}^{(2)}(\mathrm{G})$ is the value of T at equilibrium or quasi-stationarity (function of G).*

In biological terms, comparing the value of $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}$ at quasi-stationarity (representing a model with no genetic regulation) and at equilibrium allows understanding the role of genetic regulations.

Biological prediction 6 (response of ATP during fasting)
If the Conditions 1-5 are satisfied, then ATP decreases during fasting and increases during feeding. This prediction is valid for rapid (at quasi-stationarity) as well as for slow (at equilibrium) response.

Proposition 4.4. *Under the hypothesis of Prop. 4.2, then $\left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{qs} > \left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq} > 0$.*

Let us emphasize that the derivative $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}$ quantifies the energy buffering effect: the lower is this derivative, hence the lower is the variation of T for a fixed variation of G, the stronger is the energy buffering effect. Thus, we can state the following:

Biological prediction 7 (Role of genetic regulations in energy homeostasis)
Under the hypothesis of Prop. 4.2, genetic regulation reinforces the energy buffering effect.

4.3. PPAR knock-out reduces energy buffering and increases PUFA at fasting

Our qualitative model can predict the effects of various genetic perturbations. Let us consider here the effect of PPAR knock-out. Without PPAR, there is no longer a genetic control on oxidation, therefore we expect to have less energy buffering on fasting. Less obvious is what happens to the concentration of PUFA. We can predict the behavior of PUFA, under the same hypothesis as above. Let $\mathrm{F_2}^{(2)}(\mathrm{G})$ be the value of PUFA concentration as a function of G. Also, let $B_{WT,eq}$, $B_{PPAR-/-,eq}$ be the values at equilibrium in wild type and mutants of the coefficient B defined at Proposition 3.4.

Then we have:

Proposition 4.5. *If conditions 1-6 and $B_{WT,eq} > B_{PPAR-/-,eq}$ are satisfied, then*

1. $\left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq,PPAR-/-} > \left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq,WT}$,
2. $\left|\frac{d\mathrm{F_2}^{(2)}}{d\mathrm{G}}\right|_{eq,PPAR-/-} > \left|\frac{d\mathrm{F_2}^{(2)}}{d\mathrm{G}}\right|_{eq,WT}$.

Biological prediction 8 (PPAR mutants)
Under the hypotheses of Prop. 4.5 we have:

1. *PPAR knock-out reduces energy buffering.*
2. *PUFA concentration increase under fasting is stronger in PPAR knocked-out cells compared to the same increase in wild type cells.*

Comment. The condition $B_{WT,eq} > B_{PPAR-/-,eq}$ is equivalent to $B_1 \left(R_{\mathrm{F_2}}^{\mathrm{Oxi1}}\right)_{WT,eq} + B_2 \left(R_{\mathrm{F_2}}^{\mathrm{Kout}}\right)_{WT,eq} + B_4 \frac{\partial \mathrm{Oxi2}}{\partial E3}\left(\frac{\partial E3}{\partial \mathrm{F_2}}\right)_{WT,eq} > 0$, with $B_1 > 0$, $B_4 \frac{\partial \mathrm{Oxi2}}{\partial E3}\left(\frac{\partial E3}{\partial \mathrm{F_2}}\right)_{WT,eq} > 0$. This means that even if B_2 is negative the oxidation genetic control term is large enough to compensate.

Experiments on transgenic mice showed that after a 72h-fast, fatty acids concentration increases at a higher extent in PPAR knocked-out cells with respect to wild type cells [BLC⁺04]. This is coherent with the observations by Lee et al.[La04] that for the same length of fasting time the hepatic accumulation of triacylglycerol is 2.8 fold higher in PPAR knocked-out than in wild-type mice. Hence, the global behavior of fatty acids is consistent with our predictions.

Remark. Data from [La04] show a rather selective behavior among different hepatic TG PUFA in PPAR knocked-out mice: concentrations (in mg/g of liver) of α-Linolenic acid are amplified on fasting to 4-fold higher levels than in WT mice, but Arachidonic, Docosahexaenoic and Eicosapentaenoic acids are depleted to non-detectable levels in PPAR mutants. Consequently, the behavior of some important regulating PUFA in mutants can not be explained by our model, at least not within the assumptions that we have made. In fact, our model is too crude to explain the contradictory behavior of part of the

PUFA in mutants. A more complex model including more variables for PUFA and genes, could answer the questions raised by the experiment. Such a model should separate essential fatty acids from long-chain PUFA synthetized from the essential fatty acids, and include the genes involved in the synthesis of long-chain PUFA.
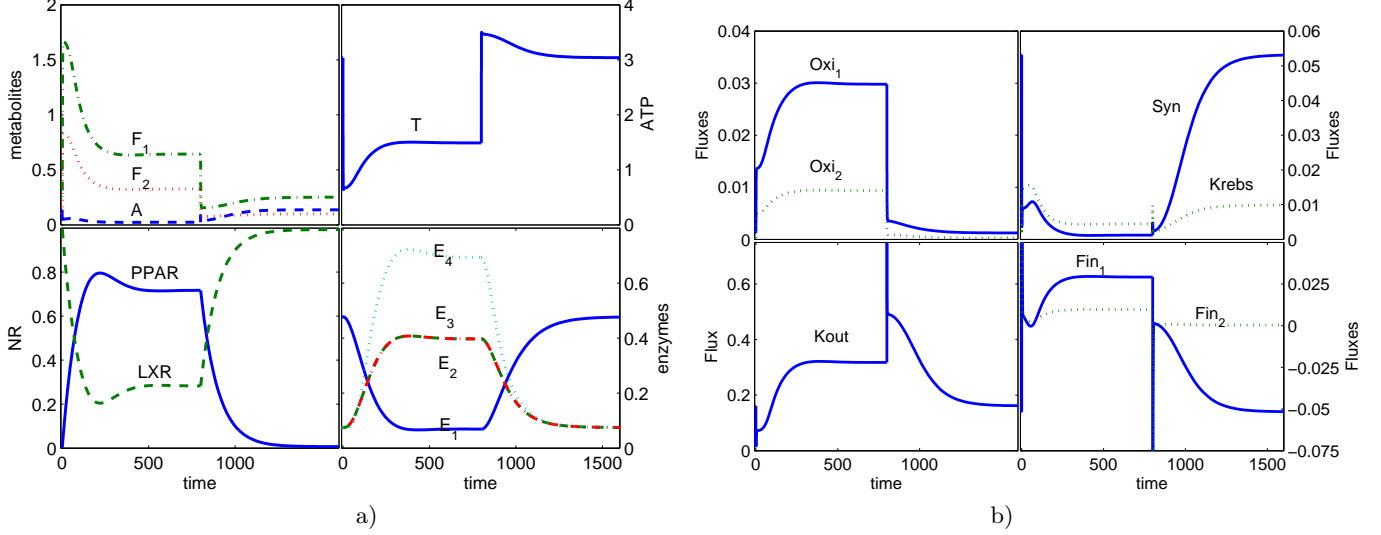

Fig. 4.1. Starving/refeeding protocol. a) Concentrations of metabolites and main regulators. Starving begins at $t = 0$ and refeeding at $t = 750$ (G = 0 for $0 < t < 750$ and G = 10 for $t < 0$, $t > 750$). Fatty acids (F_1 S/MU-FA, F_2 PUFA) increase at fasting, with overshoots, decrease at refeeding, with undershoots. Acetyl-coA A and T (ATP, energy) have the opposite behavior. By PPAR and LXR we mean the active forms of nuclear receptors. These stimulate oxidation and ketonic exits at fasting (LXR falls, PPAR raises) and synthesis at refeeding (LXR raises, PPAR falls). b) Fluxes. At fasting (G = 0) there is practically no synthesis, and the Krebs cycle functions at a very low level. The fatty acid intake fluxes Fin are positive, oxidation and ketonic exit are strong. Refeeding inverses the Oxi,Kout/Syn,Krebs flux ratios, and changes the sign of the fatty acids intake fluxes (which become negative).

4.4. Illustration of dynamics

In order to illustrate the predictions of the previous section and the dynamical behavior of the model, we move here to another level of abstraction. The forms of the functions describing how primitive fluxes depend on concentrations are chosen, including numerical constants. The choice is rather generic, by no means precise.

- **Michaelis-Menten regulation functions.** The characteristic features of those functions are the following: their value at 0 is 0; their derivative is positive and strictly decreasing to 0 at infinity. A typical analytical expression is: $f(x) = \frac{kx}{K+x}$, where k is the asymptotic value at infinity and k/K is the slope at the origin. This type of function models the dependency of an primitive flux on its substrate.
- **Repression functions.** They are positive, strictly decreasing functions, tending to 0 at infinity and have an inflexion point. A typical analytical expression is $f(x) = \frac{k}{1+Kx^a}$, where k is the maximal value (attained at 0); K controls the position of the inflexion point x_θ: $x_\theta = \left(\frac{a-1}{K(a+1)}\right)^{1/a}$. $a > 1$ (also called Hill coefficient) is the logarithmic slope at the inflexion point.
- **Activation functions.** They are positive, strictly increasing bounded functions and have an inflection point. A typical expression is $f(x) = b + \frac{kx^a}{K+x^a}$ with $K > 1$. At 0, the value is b; the function tends to $k + b$ at infinity. K controls the position of the inflexion point: $x_\theta = \left(\frac{K(a-1)}{a+1}\right)^{1/a}$. $a > 1$ is the logarithmic slope at the inflexion point.
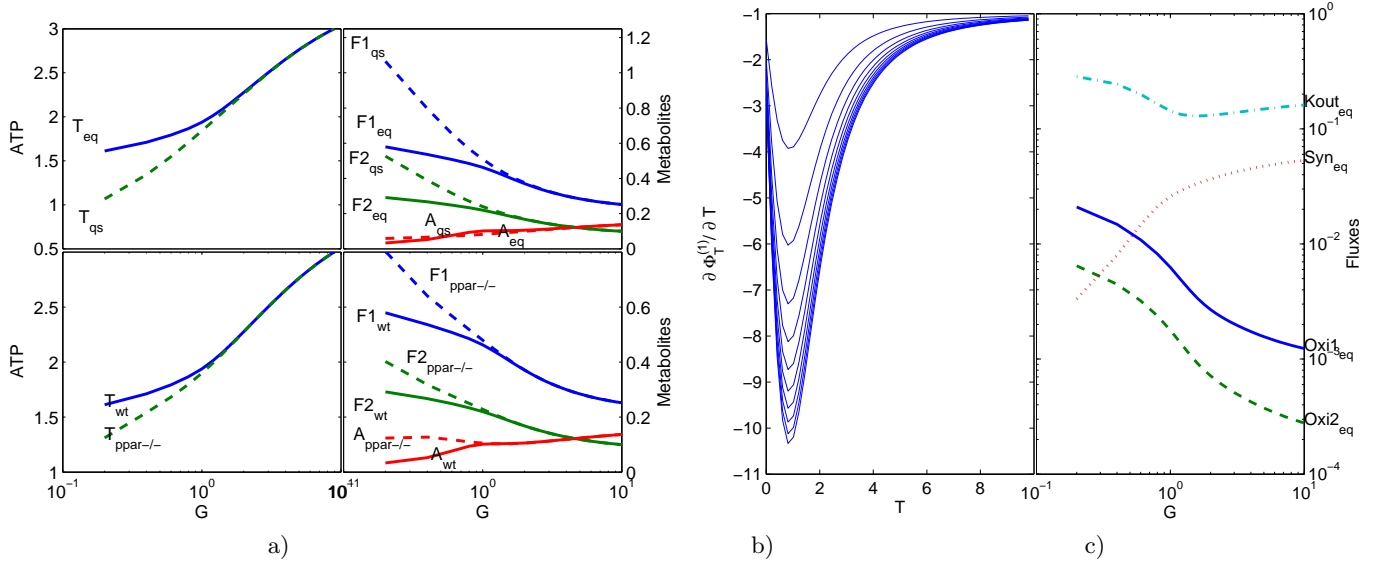
Fig. 4.2. a) Response curves for varying food (glucose G). Quasi-stationarity (qs) corresponds to passing from a conventionally chosen normal, fixed feeding level G = 10 to a smaller, variable value of G, with enzyme levels unchanged from the normal feeding equilibrium. Genetic effect on energy balance : the energy T is lower at quasistationarity (no genetic regulation). Fatty acids increase with fasting (decreasing G); this behavior is more pronounced at quasistationarity. b) The strong lipolytic condition meaning that $\frac{\partial \Phi_T^{(1)}}{\partial T} < 0$ has been plotted as a function of T, and for various values of G ($0 \leq G \leq 10$). c) Mode commutation; when food increases, the model commutes from a oxidation dominated mode to a synthesis dominated mode.
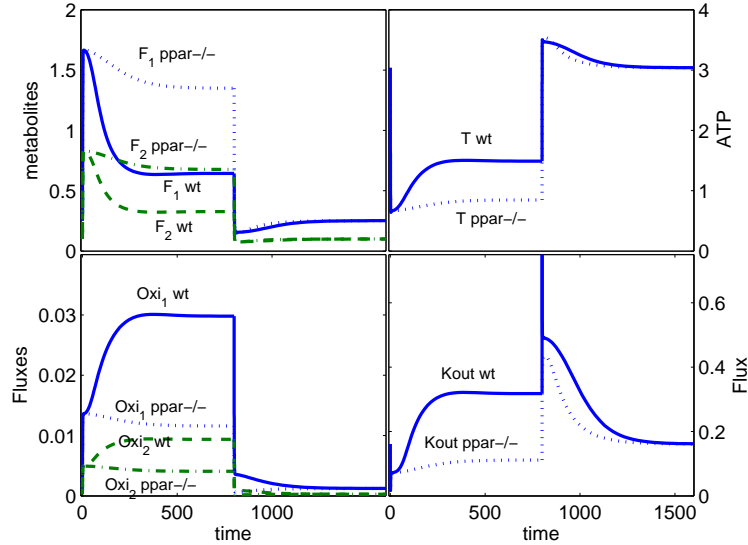
Fig. 4.3. Starving/refeeding protocol: dynamics of metabolites, energy and fluxes for a PPAR-/- mutant compared to wild-type. Fasting starts at $t = 0$, refeeding starts at $t = 750$.

– **Energy dependence functions.** Some reactions start when energy is missing and rapidly decrease to zero when energy increases. Other, like synthesis, are energy stimulated. We suppose that energetic regulation is sigmoidal, rather than hyperbolic. We use the following repressing function $f(x) = \frac{l}{L+x^2}$ and the following activating function $g(x) = \frac{lx^2}{L+x^2}$.
– **Degradation functions.** All products are supposed to degrade at a rate proportional to their concentration.
– **Consumption of ATP.** Concerning ATP, we suppose that the consumption is linear.

– **Intake/outake of fatty acids.** The case of intake/outake of fatty acids is special. Both reactions are reversible. We suppose that outake is proportional to the internal concentration of fatty acids and intake occurs only when energy is missing inside the cell.

Equations for metabolic fluxes

$$\text{Gly}(G,T) = \frac{l_{\text{Gly}}}{L_{\text{Gly}}+T^2}\frac{k_{\text{Gly}}G}{K_{\text{Gly}}+G} \qquad \text{Krebs}(A,T) = \frac{l_{\text{Krebs}}}{L_{\text{Krebs}}+T^2}\frac{k_{\text{Krebs}}A}{K_{\text{Krebs}}+A} \qquad \text{Syn}(A,T,E_1) = \frac{k_{\text{Syn}}E_1A}{K_{\text{Syn}}+A}\frac{l_{\text{Syn}}T^2}{L_{\text{Syn}}+T^2}$$

$$\text{Oxi2}(F_2,T,E_3) = \frac{l_{\text{Oxi2}}}{L_{\text{Oxi2}}+T^2}\frac{k_{\text{Oxi2}}E_3F_2}{K_{\text{Oxi2}}+F_2} \quad \text{Oxi1}(F_1,T,E_2) = \frac{l_{\text{Oxi1}}}{L_{\text{Oxi1}}+T^2}\frac{k_{\text{Oxi1}}E_2F_1}{K_{\text{Oxi1}}+F_1} \quad \text{DegT} = \delta_T T$$

$$\text{Fin1}(F_1,T) = -k_{\text{Fin1}}F_1 + \frac{l_{\text{Fin1}}}{L_{\text{Fin1}}+T^2} \quad \text{Fin2}(F_2,T) = -k_{\text{Fin2}}F_2 + \frac{l_{\text{Fin2}}}{L_{\text{Fin2}}+T^2} \quad \text{Kout}(A,E_4) = \frac{k_{\text{Kout}}E_4A}{K_{\text{Kout}}+A}$$

Equations for genetic variables All controls are positive activations except the control of F_2 on L:

$$\widetilde{\psi}_1(F_2) = \frac{k_{\text{PP}}F_2^{a_{\text{PP}}}}{K_{\text{PP}}+F_2^{a_{\text{PP}}}} + b_{\text{PP}} \quad \widetilde{\psi}_2(F_2) = \frac{k_L}{1+K_L F_2^{a_L}} \qquad \widetilde{\psi}_3(L) = \frac{k_{E_1}L^{a_{E_1}}}{K_{E_1}+L^{a_{E_1}}} + b_{E_1}$$

$$\widetilde{\psi}_4(\text{PP}) = \frac{k_{E_2}\text{PP}^{a_{E_2}}}{K_{E_2}+\text{PP}^{a_{E_2}}} + b_{E_2} \quad \widetilde{\psi}_5(\text{PP}) = \frac{k_{E_3}\text{PP}^{a_{E_3}}}{K_{E_3}+\text{PP}^{a_{E_3}}} + b_{E_3} \quad \widetilde{\psi}_6(\text{PP}) = \frac{k_{E_4}\text{PP}^{a_{E_4}}}{K_{E_4}+\text{PP}^{a_{E_4}}} + b_{E_4}$$

The final system in given in Table 4.1.

$$\begin{cases}
\frac{dA}{dt} = \frac{k_{\text{Gly}}G}{K_{\text{Gly}}+G} + n_1\frac{l_{\text{Oxi1}}}{L_{\text{Oxi1}}+T^2}\frac{k_{\text{Oxi1}}E_2F_1}{K_{\text{Oxi1}}+F_1} + n_2\frac{l_{\text{Oxi2}}}{L_{\text{Oxi2}}+T^2}\frac{k_{\text{Oxi2}}E_3F_2}{K_{\text{Oxi2}}+F_2} - \frac{l_{\text{Krebs}}}{L_{\text{Krebs}}+T^2}\frac{k_{\text{Krebs}}A}{K_{\text{Krebs}}+A} \\
\qquad - \frac{k_{\text{Kout}}E_4A}{K_{\text{Kout}}+A} - n_1\frac{k_{\text{Syn}}E_1A}{K_{\text{Syn}}+A}\frac{l_{\text{Syn}}T^2}{L_{\text{Syn}}+T^2} - \delta_A A \\
\frac{dF_1}{dt} = \frac{k_{\text{Syn}}E_1A}{K_{\text{Syn}}+A}\frac{l_{\text{Syn}}T^2}{L_{\text{Syn}}+T^2} - k_{\text{Fin1}}F_1 + \frac{l_{\text{Fin1}}}{L_{\text{Fin1}}+T^2} - \frac{l_{\text{Oxi1}}}{L_{\text{Oxi1}}+T^2}\frac{k_{\text{Oxi1}}E_2F_1}{K_{\text{Oxi1}}+F_1} - \delta_{F_1}F_1 \\
\frac{dF_2}{dt} = -k_{\text{Fin2}}F_2 + \frac{l_{\text{Fin2}}}{L_{\text{Fin2}}+T^2} - \frac{l_{\text{Oxi2}}}{L_{\text{Oxi2}}+T^2}\frac{k_{\text{Oxi2}}E_3F_2}{K_{\text{Oxi2}}+F_2} - \delta_{F_2}F_2 \\
\frac{dT}{dt} = \alpha_G\frac{l_{\text{Gly}}}{L_{\text{Gly}}+T^2}\frac{k_{\text{Gly}}G}{K_{\text{Gly}}+G} + \alpha_K\frac{l_{\text{Krebs}}}{L_{\text{Krebs}}+T^2}\frac{k_{\text{Krebs}}A}{K_{\text{Krebs}}+A} + \alpha_{O1}\frac{l_{\text{Oxi1}}}{L_{\text{Oxi1}}+T^2}\frac{k_{\text{Oxi1}}E_2F_1}{K_{\text{Oxi1}}+F_1} \\
\qquad + \alpha_{O2}\frac{l_{\text{Oxi2}}}{L_{\text{Oxi2}}+T^2}\frac{k_{\text{Oxi2}}E_3F_2}{K_{\text{Oxi2}}+F_2} - \alpha_S\frac{k_{\text{Syn}}E_1A}{K_{\text{Syn}}+A}\frac{l_{\text{Syn}}T^2}{L_{\text{Syn}}+T^2} - \delta_T T \\
\frac{d\text{PP}}{dt} = \frac{k_{\text{PP}}F_2^{a_{\text{PP}}}}{K_{\text{PP}}+F_2^{a_{\text{PP}}}} - \delta_{\text{PP}}\text{PP} + b_{\text{PP}} \\
\frac{dL}{dt} = \frac{k_L}{1+K_L F_2^{a_L}} - \delta_L L \\
\frac{dE_1}{dt} = \frac{k_{E_1}L^{a_{E_1}}}{K_{E_1}+L^{a_{E_1}}} - \delta_{E_1}E_1 + b_{E_1} \\
\frac{dE_2}{dt} = \frac{k_{E_2}\text{PP}^{a_{E_2}}}{K_{E_2}+\text{PP}^{a_{E_2}}} - \delta_{E_2}E_2 + b_{E_2} \\
\frac{dE_3}{dt} = \frac{k_{E_3}\text{PP}^{a_{E_3}}}{K_{E_3}+\text{PP}^{a_{E_3}}} - \delta_{E_3}E_3 + b_{E_3} \\
\frac{dE_4}{dt} = \frac{k_{E_4}\text{PP}^{a_{E_4}}}{K_{E_4}+\text{PP}^{a_{E_4}}} - \delta_{E_4}E_4 + b_{E_4}
\end{cases}$$

Table 4.1. A generic example for the model of the regulated metabolism.

Simulation of fasting/refeeding protocols In Fig.4.1 we have simulated a fasting/refeeding protocol. One can notice the increase of fatty acids on fasting with an overshoot (as predicted in Prop. 4.2 and its Cor.5).

The dynamics has two timescales: a quick increase up to the maximum, then a slow decrease. The concentration of Acetyl-coA is decreasing at fasting. The energy (ATP) has an abrupt fall, then it recovers slowly as a result of oxidation. The behavior of nuclear receptors correspond to what is experimentally observed [Jum04]: LXR diminishes and PPAR is amplified at fasting. This leads to variations of the enzymes: oxidation E_2, E_3 and ketone exit enzymes E_4 are amplified, the synthesis enzyme E_1 is diminished. Again, this fits with experimental observations [Jum04]. Furthermore, fluxes have textbook behavior [Sal99]. Oxidation and ketone exist occurs during fasting, while synthesis occurs during normal feeding. During fasting fatty acids enter the cell Fin1, Fin2 > 0. At normal feeding, Fin1 changes sign (de novo synthesized fatty acids exit) and Fin2 vanishes. The ketone overshoot at refeeding can be explained by enzyme inertia. The high fasting level of enzyme E_4 can not drop immediately. As large amounts of Acetyl-coA are again available, this boosts the ketone production.

The reader should be warned of a possibility not studied in this paper. Two timescales dynamics, involving the observability of the quasistationary states and the rapid overshoots and undershoots in the dynamics of metabolites and fluxes, can be avoided. We have considered here that at fasting, the glucose level falls immediately. A smooth, instead of a discontinuous decrease of glucose, is more probable

at fasting. For a slowly changing input, the system is always close to the shifting equilibrium, avoids quasistationarity and rapid transients. Because we do not study glucose dynamics and homeostasis, our model can not tell how glucose varies in time. This information should be provided by experiment.

In Fig.4.2 we have simulated response curves of various metabolites, fluxes and energy when food G is changing. It can be noticed that fatty acids concentrations decrease with food and that Acetyl-coa concentration increases with food. Energy T is increasing with food. There is a buffering effect, that preserves energy against variations of food: energy T is not zero when food G is zero. As discussed in Prop. 4.4, a strong buffering effect means a weak slope of the dependence of T on G. Genetic regulation decreases this slope, therefore increases buffering as can be seen by comparing the curves in Fig.4.2a) at quasi-stationarity and at equilibrium. The antagonistic relation between synthesis and oxidation is illustrated in Fig.4.2c): when food G decreases, the synthesis dominated regime changes to an oxidation dominated regime.

Satisfiability of the uniqueness and strong lipolytic conditions Our sufficient uniqueness conditions, allow, with small computational effort, to check the uniqueness of equilibrium for a given set of parameters. We have checked the strong lipolytic response condition for various sets of parameters. We have noticed that this condition is robust (see Fig.4.2b)).

Simulation of the effect of PPAR mutations We have modeled a PPAR mutant by considering that the enzymes E_2, E_3, E_4 controlled by PPAR have constant, unadjustable values. We have considered that the values of E_2, E_3, E_4 are those for the normal feeding equilibrium state in wild type hepatocytes. In Fig.4.3 the dynamics during a fasting/refeeding protocol are compared in the mutant and wild type case. The main feature of mutants is the difficulty to recover energy at fasting. This is the consequence of inefficient oxidation (notice the low oxidation fluxes in Fig.4.3). Similarly, in mutants the ketone production is decreased and the fatty acids increase at fasting is more pronounced just like we have predicted qualitatively in Prop. 4.5 and its Cor.8.

5. Discussion

Let us first summarize some of the characteristics of our model.

- It is an *integrative model*, because it takes into account all the main processes of carbohydrate and fatty acid metabolism in liver (glycolysis, lipogenesis, Krebs cycle, fatty acids mobilization, oxidation, ketogenesis) together with their various regulation (metabolic, genetic).
- Our model is *not distributed*: dynamical variables cope for average values in a tissue and no space information is taken into account.
- The model that we propose is a *low complexity abstraction*. It has just enough complexity to represent the basic features of metabolism in the main nutritional states. Whenever possible, complex metabolic chains of reactions were modeled as a single global reaction preserving the overall balance of products and reactants. Our main concern was to keep the model as qualitative as possible.
- *Our predictions are not dependent on specific numerical values* of kinetic constants, on specific reaction mechanisms, or on specific forms of the functions relating fluxes to concentrations. We rather replace this information by sufficient qualitative conditions that are chosen as biologically significant as possible.
- A qualitative approach has been used to discuss *response properties*. The introduction of the two types of states (quasi-stationary for rapid response and equilibrium for slow response) allowed us to distinguish between quick metabolic and slow genetic response.

Our model copes with the main experimental findings on the behavior of regulated fatty acid metabolism in hepatocytes. Under fasting, the model shifts from a synthesis dominated regime to an oxidation/lipolysis dominated regime. This shift stabilizes energy, replacing food supply by reserve consumption. At short times, the shift is performed by metabolic control of synthesis, lipolysis and oxidation. At longer times, the regulatory effect of an increase of intracellular PUFA on the nuclear receptors PPAR and LXR reinforces this control. Refeeding shifts the system in the opposite direction.

Our model is sufficiently general to apply to various higher organisms. Nevertheless, there are some biases in this model. More precisely, we have considered only hepatocytes, which is justified by the fact that in various species like chicken, rodents and humen, fatty acid synthesis and oxidation occur in the same organ (liver). This is not so for other species such as pigs, for which synthesis takes place mainly in adipocytes.

We have also proposed a methodology to build small complexity abstractions that integrate various qualitative aspects of regulated metabolism. These abstractions are by no means rigid. On the contrary, they are evolutive and can integrate new experimental results. As an example of possible evolution of the model presented here we should mention the role of various PUFA, already discussed.

6. Method

From here on, we develop the mathematical setting and arguments which lead to the results announced before.

6.1. Switches, shifts and obervability of equilibria

Equilibrium switches and shifts. The two types of equilibrium changes shift and switch can be mathematically described as follows. Let $\mathbf{Z} = (\mathbf{X}, \mathbf{Y})$ and $F(\mathbf{Z}, \mathbf{p})$, $\mathbf{Z} \in U \subset \mathbb{R}^n$, $\mathbf{p} \in I \subset \mathbb{R}$, be a differentiable vector field defined on an open set U of $\mathbb{R}^n$ depending smoothly on a parameter evolving in an open interval I. We suppose that for any $\mathbf{p}$ in a subinterval $J \subset I$, the vector field $F_{\mathbf{p}} : \mathbf{Z} \to F(\mathbf{Z}, \mathbf{p})$ admits at least a singular (equilibrium) point, that is: there exist $\mathbf{Z} \in U$ such that $F_{\mathbf{p}}(\mathbf{Z}) = 0$. We call the 0-level (equilibrium) curve:

$$\mathcal{L} = \{ (\mathbf{p}, \mathbf{Z}) \in J \times U, \, F_{\mathbf{p}}(\mathbf{Z}) = 0 \}. \tag{6.1}$$

$\mathcal{L}$ is a differentiable curve in $J \times U$. According to its shape, we get a switch or a shift. More precisely,

1. A *shift* is characterized by the following features:
   - $\mathcal{L}$ is a graph $\mathbf{Z} = L(\mathbf{p})$. Equivalently it means that for each value of $\mathbf{p} \in J$ there exist a *unique* equilibrium point,
   - $\mathcal{L}$ is a sigmoid. That is there exist a threshold value $\mathbf{p}_0$, an interval $]\mathbf{p}_0 - \delta, \mathbf{p}_0 + \delta[$, two equilibrium values $\mathbf{Z}_1$ and $\mathbf{Z}_2$ and $\epsilon > 0$ such that for $\mathbf{p} < \mathbf{p}_0 - \delta$, $|L(\mathbf{p}) - \mathbf{Z}_1| < \epsilon$ and for $\mathbf{p} > \mathbf{p}_0 + \delta$, $|L(\mathbf{p}) - \mathbf{Z}_2| < \epsilon$.

   See an illustration in Fig. 6.1.

   The sigmoid shape of the level curve has two consequences: a *"jump" effect* and *reversibility*. If we start with a certain value of the parameter $\mathbf{p}_I$, say $\mathbf{p}_I < \mathbf{p}_0 - \delta$, and increase smoothly the parameter up to a final value $\mathbf{p}_F > \mathbf{p}_0 + \delta$, we first observe an asymptotic state close to $\mathbf{Z}_1$ and a sudden "jump" to an asymptotic state close to $\mathbf{Z}_2$. We insist on that the apparent "jump" is due to the steepness of $\mathcal{L}$ at $\mathbf{p}_0$ which induces a small δ: in reality there is no discontinuity. Now if we start with the value $\mathbf{p}_F$ and decrease smoothly the parameter, we note exactly the reverse: a jump at $\mathbf{p}_0$ from an asymptotic state close to $\mathbf{Z}_2$ to an asymptotic state close to $\mathbf{Z}_1$, a property so-called reversibility.

   Notice that both properties are often used to identify shift like phenomena.
2. A *switch* has the following properties:
   - $\mathcal{L}$ is not a graph. In particular, there exist two parameter values $\mathbf{p}_0$ and $\mathbf{p}_1$ for each of which the vector field has two singularities (equilibria) and for any $\mathbf{p} \in]\mathbf{p}_0, \mathbf{p}_1[$, the vector field $F_{\mathbf{p}}$ has three singularities (one unstable and two stable). For $\mathbf{p} < \mathbf{p}_0$ or $\mathbf{p} > \mathbf{p}_1$ there is only one singularity (stable).
   - $\mathbf{p}_0$ and $\mathbf{p}_1$ are bifurcation points of saddle node type, that is an attracting and a repelling singularity collide and disappear.
   - There exist $\delta > 0$, two equilibrium values $\mathbf{Z}_1$ and $\mathbf{Z}_2$ and $\epsilon > 0$ such that for $\mathbf{p} < \mathbf{p}_0 - \delta$, $|L(\mathbf{p}) - \mathbf{Z}_1| < \epsilon$ and for $\mathbf{p} > \mathbf{p}_1 + \delta$, $|L(\mathbf{p}) - \mathbf{Z}_2| < \epsilon$.

   To understand the hysteresis effect implied by the shape of the curve $\mathcal{L}$ it is worth considering the experimental curve, that is, the curve of observed equilibria when moving the parameter. If we start with a certain value of the parameter $\mathbf{p}_I$, say $\mathbf{p}_I < \mathbf{p}_0 - \delta$, and increase smoothly the parameter up to a final value $\mathbf{p}_F > \mathbf{p}_0 + \delta$, we first observe an asymptotic state close to $\mathbf{Z}_1$ and a sudden "jump" to an asymptotic state close to $\mathbf{Z}_2$. To the contrary to the previous shift situation, this jump is a real discontinuity. Notice that the jump occurs at the parameter value p_1. Now if we start with the value $\mathbf{p}_F$ and decrease smoothly the parameter, we note a jump at $\mathbf{p}_0$ from an asymptotic state close to $\mathbf{Z}_2$ to an asymptotic state close to $\mathbf{Z}_1$. We do not have reversibility, because the jump occurs for different critical values of $\mathbf{p}$. The curves in both cases are depicted in Fig. 6.1.
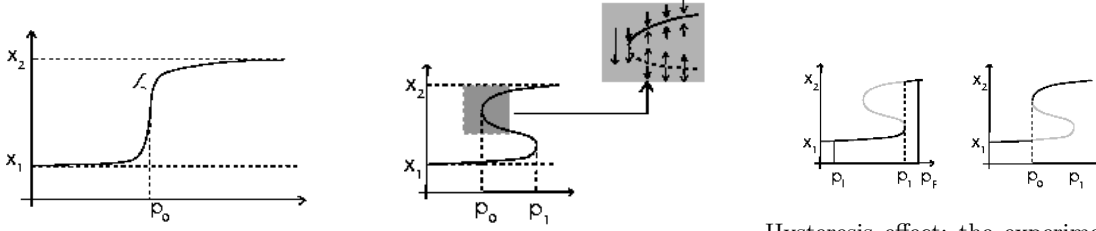
A shift: the 0-level curve is a sigmoid

A switch: the 0-level curve is not a graph. Around $\mathbf{p}_0$ and $\mathbf{p}_1$ a saddle-node bifurcation occurs.

Hysteresis effect: the experimental curves.

Fig. 6.1. Switches and shifts

Experimental curves and equilibria. Consider now the differential system:

$$\frac{d\mathbf{Z}}{dt} = \mathbf{F}(\mathbf{Z}, p), \quad \mathbf{Z} \in \mathbb{R}^n_+, p \in I \subset \mathbb{R} \tag{6.2}$$

The points on the 0-level curve of the vector field $\mathbf{F}$ are equilibria of the dynamical system (Eq.(6.2)). In the following we consider the simple situation when for any $p \in I$ there is a unique attractive equilibrium $\mathbf{Z}_0(p)$, of open attractive basin $B(p)$. Let us recall that the attraction basin consists of all $\mathcal{Z}$ such that the trajectory $\mathbf{u}(t, \mathbf{Z}, p)$ of the system (6.2), starting at $\mathbf{Z}$, approaches $\mathbf{Z}_0(p)$ for large times $|u(t, \mathbf{Z}, p) - \mathbf{Z}_0(p)| \to 0 \quad (t \to \infty)$.

A typical experimentation consists in starting in an equilibrium $\mathbf{Z}_0(p_1)$ and suddenly changing the value of the parameter p from p_1 to p_2. Suppose that the following condition is fulfilled $\mathbf{Z}_0(p_1) \in B(p_2)$. This condition is automatically fulfilled if for instance $\mathbf{Z}_0(p_2)$ is globally attractive, i.e. $B(p_2) = \mathbb{R}^n_+$. Then, the observed state $\mathbf{u}(t, \mathbf{Z}_0(p_1), p_2)$ will approach the state $\mathbf{Z}_0(p_2)$ on the 0-level curve after a long enough time. Suppose now that the 0-level curve $\mathbf{Z} = L(p)$ is such that the component $L_i(p)$ satisfies $\frac{dL_i}{dp} > 0$ for any $p \in I$. Then, it exists T such that $u_i(t, \mathbf{Z}_0(p_1), p_2) > (Z_0)_i(p_1)$ for $t > T$, meaning that we observe an increase of the component i of the state of the system between the beginning and the end of the experimentation.

Hence, *the shape of the 0-level curve informs on variations of products during an experiment.* This justifies that we can predict experimental behaviors of the system from the study of its equilibria.

Observability of quasi-stationarity. Let us suppose now that the system (6.2) has two time scales, one slow and one fast. This can be taken into account by supposing that there is a small parameter ϵ representing the fast time scales and that the system (6.2) reads $\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, p), \frac{d\mathbf{Y}}{dt} = \epsilon\mathbf{\Psi}(\mathbf{X}, \mathbf{Y}, p)$, where $\mathbf{X} \in \mathbb{R}^{n_f}, \mathbf{Y} \in \mathbb{R}^{n_s}, n_f + n_s = n$. Considering the time scale $\tau = \epsilon t$ we arrive to the more classical form:

$$\epsilon\frac{d\mathbf{X}}{d\tau} = \mathbf{\Phi}(\mathbf{Z}, p), \frac{d\mathbf{Y}}{d\tau} = \mathbf{\Psi}(\mathbf{Z}, p) \tag{6.3}$$

A result due to Tikhonov [TVS80] and reformulated by Fenichel [Fen79] implies that the system (6.3) has a remarkable behavior. Suppose that the following two conditions are satisfied:

– For fixed $\mathbf{Y} = \mathbf{Y}_0$, the system $\frac{d\mathbf{X}}{d\tau} = \mathbf{\Psi}(\mathbf{X}, \mathbf{Y}_0, p)$ has an attractor $X_0(\mathbf{Y}_0, p)$ that satisfies the relation $\mathbf{\Psi}(\mathbf{X}_0(\mathbf{Y}_0, p), \mathbf{Y}_0, p) = 0$. The equation $\mathbf{X} = \mathbf{X}_0(\mathbf{Y}_0, p)$ defined the slow manifold $\Sigma_0(p)$.
– The Jacobian matrix $D_\mathbf{X}\mathbf{\Psi}$ admits n_f eigenvalues with strictly negative real parts at $(\mathbf{X}_0(\mathbf{Y}_0, p), \mathbf{Y}_0)$.

Then the system (6.3) admits an attractive invariant manifold $\Sigma(\epsilon, p)$ close to the slow manifold $\Sigma_0(p)$. Thus, trajectories sufficiently close to the slow manifold converge to it. The smaller ϵ is, the quicker is the convergence. Notice that the slow manifold defines the quasi-stationary states. The Tikhonov-Fenichel result means that the system rapidly tends to quasi-stationarity.

6.2. Existence of equilibrium (Theorem 3.1)

The proof of Theorem 3.1 uses the following more general theorem:

Theorem 6.1. *Let $\mathbf{\Phi}(\mathbf{X}) = \mathbf{G}(\mathbf{X}) - \mathbf{\Lambda}(\mathbf{X})$ be a smooth vector field on $\mathbb{R}^n_+$ ($\mathbb{R}^n_+$ represents all the vectors of $\mathbb{R}^n$ having non-negative coordinates) such that :*

1. $\mathbf{G}$ *is bounded,*
2. *For all $\mathbf{X} = (X_1, \ldots, X_n)$ such that $X_i = 0$ and $X_j \neq 0$ for all $j \neq i$, $\mathbf{G}$ satisfies $G_i(\mathbf{X}) > 0$,*
3. $\mathbf{\Lambda} = (\Lambda_1(X_1), \ldots, \Lambda_n(X_n)) : \mathbb{R}^n_+ \to \mathbb{R}^n_+$, *and Λ_i are differentiable and satisfy $\Lambda_i(0) = 0$ and $\lim_{\|\mathbf{X}\| \to +\infty} \Lambda_i(\mathbf{X}) = +\infty$, for all $1 \leq i \leq n$.*

Then the equation $\mathbf{\Phi}(\mathbf{X}) = 0$ has at least one solution in $\mathbb{R}^n_+$.

The proof of the Theorem is based on the following standard mathematical lemma.

Lemma 6.1. *Let D be a smooth ball in $\mathbb{R}^n$ and let S be the boundary of D. Let Φ be a differentiable vector field defined on a neighborhood of D. If $\mathbf{\Phi}$ points inward D at any point of S then $\mathbf{\Phi}$ admits a zero in the interior of D.*

Sketch of the proof of Lemma 6.1. By the Poincaré-Hopf formula a sufficient condition for having a zero in the interior of D is to have a non-zero index for the vector field on S. Since $\mathbf{\Phi}$ points inward D on S, we can construct a smooth change of variables which conjugates $\mathbf{\Phi}$ on a neighborhood of D to a vector field $\mathbf{\Phi}'$ defined on a neighborhood of the unit n−ball $\mathbf{B}^n$, such that on a neighborhood of the unit n−sphere S^n, $\mathbf{\Phi}'$ coincides with the radial vector field $\mathbf{X} \mapsto -\mathbf{X}$. For this vector field $\mathbf{\Phi}'$, we can compute its index, which is 1 or -1 according to the parity of n. The Lemma is proved since the index is a differential invariant. ∎

Proof of Theorem 6.1. From Lemma 6.1, it is enough to find a smooth ball in the positive orthant on the boundary of which the vector field $\mathbf{\Phi}$ points inwards.

For $R > 0$, let us consider the intersection domain of the closed n-ball of radius R with the positive orthant: $\Delta = \{\mathbf{X} \in \mathbb{R}^n_+, \|\mathbf{X}\| \leq R\}$. This domain is a topological ball; let us denote Σ its boundary. If $\mathbf{X} \in \Sigma$ and none of its components is 0, then for R large enough, $\mathbf{\Phi}(\mathbf{X})$ points inward Δ, because G is bounded and $\Lambda_i(\mathbf{X})$ tend to infinity with $\mathbf{X}$, hence $\Phi_i(\mathbf{X}) < 0$, for all $1 \leq i \leq n$. On the other hand, if only one of the components of $\mathbf{X}$ is 0, then by hypothesis (2), $\mathbf{\Phi}(\mathbf{X})$ points inward Δ. Since the set of points where the property of pointing inwards is open, we can find a smooth ball D contained in Δ and sufficiently close to it, such that on the boundary of D, the $\mathbf{\Phi}$ points inward D. ∎

Actually, the Theorem implies naturally a stronger result which will be useful in the proof of uniqueness of equilibrium.

Corollary 6.1. *Under the hypotheses of Theorem 6.1, let $\mathbf{X} = (\mathbf{X_1}, \mathbf{X_2})$ be any partition of the variables. We write $\Phi_1(\mathbf{X_1}, \mathbf{X_2})$ for the projection of $\Phi(\mathbf{X_1}, \mathbf{X_2})$ in the vector space spanned by the coordinates of $\mathbf{X_1}$. Given $\mathbf{X_2}$, the system of equations $\Phi_1(\mathbf{X_1}, \mathbf{X_2}) = 0$, where $\mathbf{X_2}$ is considered as a constant parameter vector, admits a solution in $\mathbf{X_1}$ with non negative entries.*

Proof. Theorem 6.1 applies to the vector field $\Phi_1(\mathbf{X_1}, \mathbf{X_2})$, where $\mathbf{X_2}$ is considered as a constant parameter vector, since Φ_1 satisfies the hypotheses of the Theorem 6.1 as soon as Φ satisfies them. ∎

Proof of Theorem 3.1. The proof runs by applying Theorem 6.1 to the following vectors:

$$\mathbf{\Lambda} = \begin{pmatrix} \mathrm{DegF}_1 \\ \mathrm{DegF}_2 \\ \mathrm{DegT} \\ \mathrm{DegA} \end{pmatrix} \qquad \mathbf{G} = \begin{pmatrix} \mathrm{Syn}_{\mathrm{peq}} - \mathrm{Oxi1}_{\mathrm{peq}} + \mathrm{Fin1} \\ -\mathrm{Oxi2} + \mathrm{Fin2} \\ \alpha_{\mathrm{G}}\mathrm{Gly} - \alpha_S Syn + \alpha_{\mathrm{K}}\mathrm{Krebs} + \alpha_{\mathrm{O1}}\mathrm{Oxi1} + \alpha_{\mathrm{O2}}\mathrm{Oxi2} \\ \mathrm{Gly} + n_1\mathrm{Oxi1} + n_2\mathrm{Oxi2} - \mathrm{Krebs} - \mathrm{Kout} - n_1\mathrm{Syn} \end{pmatrix}$$

Notice that equilibrium states of the implicit models are the zeroes of the vector field $\mathbf{\Phi}(\mathbf{X}) = \mathbf{G}(\mathbf{X}) - \mathbf{\Lambda}(\mathbf{X})$.

Let us verify hypotheses of Theorem 6.1. First, $\mathbf{G}$ is differentiable because all fluxes are differentiable. Then, $\mathbf{G}$ is bounded because it is composed of primitive fluxes which saturate at high concentrations of metabolites (Condition 3). Finally, to verify the condition $G_i(\ldots, X_i = 0, \ldots) > 0$, it is enough to notice that each coordinate can be decomposed into the difference of the fluxes which produce the variable and the fluxes which consume the variable. The second ones are zero when the variable is zero by zero substrate effect (Condition 3). The sum of the producing fluxes is strictly positive by recovery effect (Condition 3).

The assumptions on $\mathbf{\Lambda}$ are satisfied by linearity of degradation terms and by unboundedness of ATP consumption term (Condition 3). ∎

6.3. Box reduction of systems of non-linear equations

Let $\mathbf{\Phi} : \mathbb{R}^n \times \Delta \to \mathbb{R}^n$, where Δ is a compact subset of $\mathbb{R}^q$, be a differentiable vector field. $\mathbf{\Phi}$ defines the following system of linear equations parametrized by p:

$$\mathcal{S} : \mathbf{\Phi}(\mathbf{X}, \mathbf{p}) = 0 \qquad (6.4)$$

Box of a system of equations. We call box of the system (6.4) a subset $\mathbf{X}^{(i)}$ of the set of variables $\mathbf{X}$, such that $\mathbf{X} = (\mathbf{X}^{(i)}, \mathbf{X}^{(e)})$ is a partition of the set of variables. The variables $\mathbf{X}^{(i)}, \mathbf{X}^{(e)}$ are called internal and external variables, respectively. To each partition of the variables, let us consider the corresponding partition of the vector field components $\mathbf{\Phi} = (\mathbf{\Phi}^{(i)}, \mathbf{\Phi}^{(e)})$.

We call *box equilibration* the elimination of internal variables from the equations defined by the internal part of the vector field: $\mathbf{\Phi}^{(i)}(\mathbf{X}^{(i)}, \mathbf{X}^{(e)}, p) = 0$.

Sequence of box equilibration. After a box equilibration the internal variables can be expressed as functions of the external variables. A *sequence of box equilibrations* is the finite iteration of the following operations:

1. Define $\mathbf{X}_1 = \mathbf{X}$ and $\mathbf{\Phi_1}(\mathbf{X}_1, \mathbf{p}) = \mathbf{\Phi}(\mathbf{X}, \mathbf{p})$, $\mathcal{D}_1(\mathbf{p}) = \mathbb{R}^n$.
2. At k-th iteration, divide the variables and the vector field components into internal and external parts $\mathbf{X}_k = (\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)})$, $\mathbf{\Phi}_k = (\mathbf{\Phi}_k^{(i)}, \mathbf{\Phi}_k^{(e)})$.
3. If the external part is not empty then:
   - solve $\mathbf{\Phi}_k^{(i)}(\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)}, \mathbf{p}) = 0$ with the constraint $(\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)}) \in \mathcal{D}_k(\mathbf{p})$ and express the internal variables as functions of the external variables $\mathbf{X}_k^{(i)} = \mathcal{M}_\mathbf{k}(\mathbf{X}_\mathbf{k}^{(\mathbf{e})}, \mathbf{p})$. Notice that the solution might not be unique, that is $\mathcal{M}_\mathbf{k}$ is not necessarily univocal. We restrict our discussion to the case when the number of solutions is finite and bounded, such as for polynomial systems. Also, notice that one has a solution for $\mathbf{X}_\mathbf{k}^{(\mathbf{e})}$ in a maximal domain $\mathcal{D}_{k+1}(\mathbf{p})$. If $\mathcal{D}_{k+1}(\mathbf{p})$ is empty then stop: there is no solution.
   - define $\mathbf{X_{k+1}} = \mathbf{X}_\mathbf{k}^{(\mathbf{e})}$, and $\mathbf{\Phi_{k+1}} = \mathbf{\Phi}_\mathbf{k}^{(\mathbf{e})}(\mathcal{M}_\mathbf{k}(\mathbf{X}_\mathbf{n}^{(\mathbf{e})}, \mathbf{p}), \mathbf{X}_\mathbf{n}^{(\mathbf{e})}, \mathbf{p})$.
4. If the external part is empty then solve $\mathbf{\Phi}_k^{(i)}(\mathbf{X}_k^{(i)}, \mathbf{p}) = 0$ and stop. Conventionally, in this case $\mathcal{D}_{k+1}(\mathbf{p})$ is considered non-empty iff the equation has a solution.
5. go to step 2.

A sequence of box equilibrations is *complete* if all components are equilibrated i.e.

$$\mathbf{X} = \mathbf{X}_1^{(i)} \oplus \mathbf{X}_2^{(i)} \oplus \ldots \oplus \mathbf{X}_{N_b}^{(i)}.$$

After a complete sequence of box equilibrations one should be able to express metabolite levels as functions of the external parameters: $\mathbf{X} = \mathcal{M}(\mathbf{p})$, where $\mathcal{M}$ results from a composition of the functions $\{\mathcal{M}_\mathbf{k}\}_{k=1,N_b}$.

An example of (incomplete) sequence of box equilibrations is the reduction (at equilibrium) of genetic variables in the mixed metabolic/genetic differential system. There is only one box whose internal variables are the genetic variables $\mathbf{Y}$. These are eliminated from the equations $\psi(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0$. The functions $\mathcal{M}_1(\mathbf{X}, \mathbf{p})$ are $\mathbf{Y}^{peq}(\mathbf{X}, \mathbf{p})$, and the reduced fluxes are $\mathbf{\Phi}_2(\mathbf{X}, \mathbf{p}) = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}^{peq}(\mathbf{X}, \mathbf{p}), \mathbf{p})$.

Existence and uniqueness of solutions.

Box equilibrations perform nothing else than the substitution method for non-linear systems of equations. The existence and properties of solutions relatively to box equilibrations are straightforward.

Proposition 6.1. – *A solution of the system (6.4) exists for a value of the parameter p if there is a complete sequence of box equilibrations with non-empty domains $\mathcal{D}_{k+1}(\mathbf{p})$.*
  – *The function $\mathcal{M}$ is univocal (to one $\mathbf{p}$ corresponds a single value of $\mathcal{M}$) if all the domains $\mathcal{D}_{k+1}(\mathbf{p})$ are non-empty and each one of the function $\mathcal{M}_\mathbf{k}$ is univocal on its maximal domain $\mathcal{D}_{k+1}(\mathbf{p})$ for a complete sequence of box equilibrations.*

This property is useful to prove the existence and uniqueness of solutions of systems of non-linear equations. It is enough to choose a complete sequence of box equilibrations and to show that at each step the functions $\mathcal{M}_\mathbf{k}$ are univocal on non-empty domains $\mathcal{D}_{k+1}(\mathbf{p})$.

It is difficult to give a "only if" version of the property. Indeed, even if we find a box such that the equations $\mathbf{\Phi}_k^{(i)}(\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)}, \mathbf{p}) = 0$ have multiple solutions in $\mathbf{X}_k^{(i)}$ it is not excluded that some of these solutions are incompatible with the rest of the equations: after all the box equilibrations we may still have an unique solution.

Sketch of the proof of Proposition 3.2 and Theorem 3.2. According to Proposition 6.1 a sufficient condition for uniqueness of equilibrium is to find a complete sequence of box equilibrations for the state equations of metabolic variables (Eq.(2.3)). To simplify notations, in this section we write the reduced fluxes without the subscripts peq, gnr. Notice that in order to get the state equations a first box elimination of the genetic variables (shown to be univocal) has already been performed at equilibrium. For genetically non-regulated states, the reduction of the genetic variables has been performed by replacing them by constants.

Let us consider a box decomposition corresponding to the following partition of variables:

– Box 1: $\{A, F_1, F_2\}$.
– Box 2: $\{T\}$.

The main steps of the proof are the following:

– Step 1: the equilibration equations for Box 1 with internal variables A, F_1, F_2 is:

$$\Phi_A(G, A, F_1, F_2, T) = 0 \tag{6.5}$$
$$\Phi_{F_1}(A, F_1, F_2, T) = 0 \tag{6.6}$$
$$\Phi_{F_2}(F_2, T) = 0 \tag{6.7}$$

In Lemma 6.3, we prove that for all $(G, T) \in [0, G_{max}] \times \mathbb{R}_+$, this system has a unique solution $(A^{(1)}(G, T), F_1^{(1)}(G, T), F_2^{(1)}(G, T)) \in \mathbb{R}_+^3$, which ends the proof of Proposition 3.2.
– Step 2: the equilibration equation for Box 2 with internal variable T is:

$$\Phi_T(G, A^{(1)}(G, T), F_1^{(1)}(G, T), F_2^{(1)}(G, T), T) = 0 \tag{6.8}$$

In Lemma 6.4, we shall provide a sufficient condition for this equation to have a unique positive solution $T = T^{(2)}(G)$. The unique solution of the state equations is thus $A = A^{(2)}(G) = A^{(1)}(G, T^{(2)}(G))$, $F_1 = F_1^{(2)}(G) = F_1^{(1)}(G, T^{(2)}(G))$, $F_2 = F_2^{(2)}(G) = F_2^{(1)}(G, T^{(2)}(G))$. This solution depends on being at equilibrium or at quasi-stationarity, because the expressions of the reduced fluxes Φ_A, Φ_{F_1}, Φ_{F_2} depend on being at equilibrium or at quasi-stationarity.
– Step 3: finally, we show that the strong lipolytic condition is the sufficient condition of Lemma 6.4.

All the formal manipulations of this sequence where performed using Wolfram Research Mathematica version 5.2 software.

To treat the case of Box 1, we use the following result which is a direct consequence of Gale-Nikaido-Inada theorem [Par83]. This theorem can be seen as a generalization to higher dimensions of the monotonicity of functions on $\mathbb{R}$. Let us recall that a principal minor of a matrix $M = (m_{i,j})_{i,j \in \{1,\dots,n\}}$ is defined as $\det M_I$, where $I \subset \{1, \dots, n\}$ and $M_I = (m_{i,j})_{i,j \in I}$.

Lemma 6.2 (Gale-Nikaido). *If $(x, y, z) \rightarrow (f_x, f_y, f_z)$ is a differentiable mapping from $\mathbb{R}_+^3$ to $\mathbb{R}^3$, of Jacobian J, such that all the principal minors of $-J$ are positive, then this mapping is globally univalent. In particular the system $f_x(x, y, z) = 0$, $f_y(x, y, z) = 0$, $f_z(x, y, z) = 0$ has a unique solution if a solution exists.*

Lemma 6.3. *For all $(G, T) \in [0, G_{max}] \times \mathbb{R}_+$, the system of Eqs. (6.5),(6.6) and (6.7) has a unique solution in $(A, F_1, F_2) \in \mathbb{R}_+^3$, both at genetic partial equilibrium and at fixed genetic variables . This expresses A, F_1, F_2 as univocal functions of G and T, denoted by $A^{(1)}(G, T)$, $F_1^{(1)}(G, T)$ and $F_2^{(1)}(G, T)$.*

Proof. We apply Corollary 6.1 to prove that the system of equations (6.5), (6.6) and (6.7) has a solution for every fixed (G, T).

Let us consider the mapping $(A, F_1, F_2) \rightarrow (\Phi_A, \Phi_{F_1}, \Phi_{F_2})$ on $\mathbb{R}_+^3$. To prove the uniqueness of the solution to the system, we apply Lemma 6.2 to this mapping by ensuring that all the principal minors of $-J^{(1)}$ are positive, where $J^{(1)}$ is the Jacobian of this mapping:

$$J^{(1)} = \begin{pmatrix} -\chi_A^{tot} & \frac{\partial \Phi_A}{\partial F_1} & \frac{\partial \Phi_A}{\partial F_2} \\ \frac{\partial \Phi_{F_1}}{\partial A} & -\chi_{F_1}^{tot} & \frac{\partial \Phi_{F_1}}{\partial F_2} \\ 0 & 0 & -\chi_{F_2}^{tot} \end{pmatrix} = \begin{pmatrix} -\chi_A^{tot} & n_1 \chi_{F_1}^{Oxi1} & n_2 R_{F_2}^{Oxi2} + n_1 R_{F_2}^{Oxi1} + n_1 R_{F_2}^{Syn} - R_{F_2}^{Kout} \\ \chi_A^{Syn} & -\chi_{F_1}^{tot} & -R_{F_2}^{Oxi1} - R_{F_2}^{Syn} \\ 0 & 0 & -\chi_{F_2}^{tot} \end{pmatrix}.$$

The principals minors of $-J^{(1)}$ are all positive: $\chi_A^{tot} > 0$, $\chi_A^{tot}\chi_{F_1}^{tot} - n_1\chi_A^{Syn}\chi_{F_1}^{Oxi1} = \chi_A^{tot}\chi_{F_1}^{tot}(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1}) > 0$, $\chi_{F_2}^{tot}\chi_A^{tot}\chi_{F_1}^{tot}(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1}) > 0$, as a consequence of Prop. 3.3. This is valid both at genetic partial equilibrium and at fixed genetic variables .

Hence, Lemma 6.2 applies, so that there exist functions $A^{(1)}(G,T)$, $F_1^{(1)}(G,T)$ and $F_2^{(1)}(G,T)$ that are the unique solutions of the system of Eqs. (6.5), (6.6) and (6.7) for each (G,T). These functions are differentiable on $\mathbb{R}_+^2$ by the implicit function theorem. ∎

Proof of Proposition 3.2. Proposition 3.2 derives directly from Lemma 6.3. ∎

Lemma 6.4. *Eq.(6.8) has a unique solution in* T *as soon as the following inequality holds for every* $(G,T) \in [0, G_{max}] \times \mathbb{R}_+$:

$$\frac{\partial \Phi_T}{\partial T} + (\alpha_K\chi_A^{Krebs} - \alpha_S\chi_A^{Syn})\frac{\partial A^{(1)}}{\partial T} + \alpha_{O1}\chi_{F_1}^{Oxi1}\frac{\partial F_1^{(1)}}{\partial T} + (\alpha_{O1}R_{F_2}^{Oxi1} + \alpha_{O2}R_{F_2}^{Oxi2} + \alpha_S R_{F_2}^{Syn})\frac{\partial F_2^{(1)}}{\partial T} < 0. \quad (6.9)$$

Proof. Let $\Phi_T^{(1)}(G,T) = \Phi_T(G, A^{(1)}(G,T), F_1^{(1)}(G,T), F_2^{(1)}(G,T), T)$. The biological hypotheses imply that Theorem 3.1 applies that is, for every G, there exists $(a, f_1, f_2, t) \in \mathbb{R}_+^4$ such that

$$\Phi_A(a, f_1, f_2, t) = \Phi_{F_1}(a, f_1, f_2, t) = \Phi_{F_2}(f_2, t) = \Phi_T(G, a, f_1, f_2, t) = 0.$$

By uniqueness in the previous Lemmas, we get $a = A^{(1)}(G,t)$, $f_1 = F_1^{(1)}(G,t)$ and $f_2 = F_2^{(1)}(G,t)$. Hence $\Phi_T^{(1)}(G,t) = 0$ and the function $\Phi_T^{(1)}(G,T)$ has a root in T for every G.

Moreover, the function $\Phi_T^{(1)}$ is differentiable on $\mathbb{R}_+^2$. From the definition of the function $\Phi_T^{(1)}$ it follows:

$$\frac{\partial \Phi_T^{(1)}}{\partial T} = \frac{\partial \Phi_T}{\partial T} + (\alpha_K\chi_A^{Krebs} - \alpha_S\chi_A^{Syn})\frac{\partial A^{(1)}}{\partial T} + \alpha_{O1}\chi_{F_1}^{Oxi1}\frac{\partial F_1^{(1)}}{\partial T} + (\alpha_{O1}R_{F_2}^{Oxi1} + \alpha_{O2}R_{F_2}^{Oxi2} + \alpha_S R_{F_2}^{Syn})\frac{\partial F_2^{(1)}}{\partial T} \quad (6.10)$$

Hence if the inequality 6.9 is satisfied, then $\frac{\partial \Phi_T^{(1)}}{\partial T}$ is negative. In other words, $\Phi_T^{(1)}$ is monotonic so that it has a unique zero. ∎

Proof of Theorem 3.2 Theorem 3.2 derives directly from Lemma 6.4. ∎

Lemma 6.5. *The derivatives* $\frac{\partial A^{(1)}}{\partial T}$, $\frac{\partial F_1^{(1)}}{\partial T}$, $\frac{\partial F_2^{(1)}}{\partial T}$ *can be expressed by means of fluxes and of control coefficients in the following way:*

$$-\frac{\det(J^{(1)})}{\chi_{F_1}^{tot}}\frac{\partial A^{(1)}}{\partial T} = \chi_{F_2}^{tot}\{R_T^{Gly} - R_T^{Krebs} + n_2[\rho_{F_2}^{Oxi2}R_T^{Fin2} + (1 - \rho_{F_2}^{Oxi2})R_T^{Oxi2}] + n_1[(1 - \rho_{F_1}^{Oxi1})(R_T^{Oxi1} + R_T^{Syn}) +$$
$$\rho_{F_1}^{Oxi1}R_T^{Fin1}]\} + (R_T^{Fin2} - R_T^{Oxi2})[-R_{F_2}^{Kout} + n_1(R_{F_2}^{Oxi1} + R_{F_2}^{Syn})(1 - \rho_{F_1}^{Oxi1})]$$

$$-\frac{\det(J^{(1)})}{\chi_A^{tot}}\frac{\partial F_1^{(1)}}{\partial T} = \chi_{F_2}^{tot}\{\rho_A^{Syn}(R_T^{Gly} - R_T^{Krebs} + n_2R_T^{Oxi2}) + n_1[R_T^{Fin1} - (1 - \rho_A^{Syn})(R_T^{Oxi1} + R_T^{Syn})]\}$$
$$+ (R_T^{Oxi2} - R_T^{Fin2})[n_1(R_{F_2}^{Oxi1} + R_{F_2}^{Syn}) - n_2R_{F_2}^{Oxi2} + \rho_A^{Syn}R_{F_2}^{Kout}]$$

$$\frac{\partial F_2^{(1)}}{\partial T} = (\chi_{F_2}^{tot})^{-1}(R_T^{Oxi2} - R_T^{Fin2}) \quad (6.11)$$

where $-\det(J^{(1)}) = \chi_{F_2}^{tot}\chi_A^{tot}\chi_{F_1}^{tot}(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1})$.

Proof. The lemma follows straightforwardly from

$$\frac{\partial}{\partial T}\begin{pmatrix} A^{(1)} \\ F_1^{(1)} \\ F_2^{(1)} \end{pmatrix} = -(J^{(1)})^{-1}\frac{\partial}{\partial T}\begin{pmatrix} \Phi_A \\ \Phi_{F_1} \\ \Phi_{F_2} \end{pmatrix}.$$

∎

Lemma 6.6.

$$\frac{\partial \Phi_T^{(1)}}{\partial T} = [A(R_T^{Oxi1} - R_T^{Fin1}) + B(R_T^{Oxi2} - R_T^{Fin2}) + C - D]/[n_1(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1})] \qquad (6.12)$$

where A, B, C, D are combinations of control parameters defined in Eq. (3.2).

Furthermore, if the stoechiometric condition 5 is fulfilled, then $X > 0, A > 0, B_1 > 0, B_4 > 0, C > 0, D > 0$.

Proof. The proof is a lengthy but straightforward formal manipulation of Eqs.(6.10),(6.11). We have gathered control coefficients into as large as possible positive combinations.

As an illustration of how the stoechiometric condition was used let us consider the sign of D. From $\alpha_{O1} > \alpha_S$, $X/n_1 - \alpha_{O1}(1 - \rho_A^{Syn}) = (\alpha_{O1} - \alpha_S)\rho_A^{Syn} + n_1\alpha_K\rho_A^{Krebs} > 0$, $\rho_A^{Syn} \le 1$, it follows that $D > 0$. ∎

Proof of Proposition 3.4. It follows directly from Lemma 6.6. ∎

Lemma 6.7. *Let $T = T^{(2)}(G)$ be the solution of the equation (6.8). If the strong lipolytic response condition and the stoechimetric conditions are satisfied then $\frac{dT^{(2)}}{dG} > 0$.*

Proof. The partial derivatives with respect to G of the metabolites after equilibration of the box 1 can be obtained from:

$$\frac{\partial}{\partial G}\begin{pmatrix} A^{(1)} \\ F_1^{(1)} \\ F_2^{(1)} \end{pmatrix} = -(J^{(1)})^{-1}\frac{\partial}{\partial G}\begin{pmatrix} \Phi_A \\ \Phi_{F_1} \\ \Phi_{F_2} \end{pmatrix} = -(J^{(2)})^{-1}\begin{pmatrix} R_G^{Gly} \\ 0 \\ 0 \end{pmatrix} = \frac{R_G^{Gly}}{\chi_A^{tot}\chi_{F_1}^{tot}(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1})}\begin{pmatrix} \chi_{F_1}^{tot} \\ \chi_A^{Syn} \\ 0 \end{pmatrix}.$$

From this and from the definition of $\Phi_T^{(1)}$ it follows

$$\frac{\partial \Phi_T^{(1)}}{\partial G} = \frac{R_G^{Gly}}{n_1\chi_A^{tot}(1 - \rho_A^{Syn}\rho_{F_1}^{Oxi1})}\{n_1\alpha_K\chi_A^{Krebs} + \chi_A^{tot}[n_1\alpha_G(1 - \rho_{F_1}^{Oxi1}\rho_A^{Syn}) + \alpha_{O1}\rho_{F_1}^{Oxi1}\rho_A^{Syn} - \alpha_S\rho_A^{Syn}]\} \qquad (6.13)$$

If $\alpha_S < \alpha_{O1} < n_1\alpha_G$ (which is true by the stoechiometry condition 5), then $0 \le \alpha_{O1}(1 - \rho_A^{Syn}) = \alpha_{O1}(1 - \rho_{F_1}^{Oxi1}\rho_A^{Syn}) + \alpha_{O1}\rho_{F_1}^{Oxi1}\rho_A^{Syn} - \alpha_{O1}\rho_A^{Syn} < n_1\alpha_G(1 - \rho_{F_1}^{Oxi1}\rho_A^{Syn}) + \alpha_{O1}\rho_{F_1}^{Oxi1}\rho_A^{Syn} - \alpha_S\rho_A^{Syn}$, and consequently $\frac{\partial \Phi_T^{(1)}}{\partial G} > 0$.

The strong lipolytic response condition is equivalent to having $\frac{\partial \Phi_T^{(1)}}{\partial T} < 0$. If this is satisfied, then $\frac{dT^{(2)}}{dG} = -\left(\frac{\partial \Phi_T^{(1)}}{\partial G}\right)/\left(\frac{\partial \Phi_T^{(1)}}{\partial T}\right) > 0$. ∎

Proof of Proposition 4.3. It follows directly from Lemmas 6.7. ∎

Lemma 6.8. *Let $F_2^{(2)}(G) = F_2^{(1)}(G, T^{(2)}(G))$. If the strong lipolytic condition is satisfied, then the sign of $\frac{dF_2^{(2)}}{dG}$ is equal to the sign of $R_T^{Oxi2} - R_T^{Fin2}$.*

Proof. The chain rule gives

$$\frac{dF_2^{(2)}}{dG} = \frac{\partial F_2^{(1)}}{\partial G} + \frac{\partial F_2^{(1)}}{\partial T}\frac{dT^{(2)}}{dG}.$$

From the proof of Lemma 6.7, $\frac{\partial F_2^{(1)}}{\partial G} = 0$. It follows from Lemma 6.5 that the sign of $\frac{\partial F_2^{(1)}}{\partial T}$ is the same as the sign of $R_T^{Oxi2} - R_T^{Fin2}$. Moreover, if the strong lipolytic condition and the stoechimetric condition are satisfied, then $\frac{dT^{(2)}}{dG} > 0$. ∎

Proof of Proposition 4.1. It follows directly from Lemmas 6.7,6.8. ∎

Proof of Propositions 4.2 and 4.4. The differences between equilibrium and quasi-stationarity occur at two levels:

1. At quasi-stationarity F_2 does not regulate the genetic variables:

$$\left(R_{F_2}^{Syn}\right)_{qs} = \left(R_{F_2}^{Oxi1}\right)_{qs} = \left(R_{F_2}^{Kout}\right)_{qs} = 0. \qquad (6.14)$$

2. At quasi-stationarity the control of F_2 on its oxidation is only a metabolic substrate effect. Genetic control is added at equilibrium. We have $R_{F_2}^{\text{Oxi2}} = \frac{\partial \text{Oxi2}}{\partial F_2} + \frac{\partial E_3}{\partial F_2}\frac{\partial \text{Oxi2}}{\partial E_3}$ with $\frac{\partial \text{Oxi2}}{\partial E_3} > 0$, and $\chi_{F_2}^{tot} = R_{F_2}^{\text{Oxi2}} - \frac{\partial \text{Fin2}}{\partial F_2}$, $\rho_{F_2}^{\text{Oxi2}} = (1 - \frac{\frac{\partial \text{Fin2}}{\partial F_2}}{R_{F_2}^{\text{Oxi2}}})^{-1}$, with $\frac{\partial \text{Fin2}}{\partial F_2} < 0$. Furthermore, $\left(\frac{\partial E_3}{\partial F_2}\right)_{eq} > 0$ and $\left(\frac{\partial E_3}{\partial F_2}\right)_{qs} = 0$. Hence:

$$\left(R_{F_2}^{\text{Oxi2}}\right)_{eq} > \left(R_{F_2}^{\text{Oxi2}}\right)_{qs}, \quad \left(\chi_{F_2}^{tot}\right)_{eq} > \left(\chi_{F_2}^{tot}\right)_{qs}, \quad \left(\rho_{F_2}^{\text{Oxi2}}\right)_{eq} > \left(\rho_{F_2}^{\text{Oxi2}}\right)_{qs}. \tag{6.15}$$

We deduce from Eq.(6.13) that $\frac{\partial \Phi_T^{(1)}}{\partial G}$ is the same at equilibrium and at quasi-stationarity. From Lemma 6.6, it follows :

$$\frac{\partial \Phi_T^{(1)}}{\partial T} = \mathcal{R} - \frac{B}{n_1(1-\rho_A^{\text{Syn}}\rho_{F_1}^{\text{Oxi1}})}(R_T^{\text{Fin2}} - R_T^{\text{Oxi2}}), \tag{6.16}$$

where $\mathcal{R}$ is a term not changing from quasi-stationarity to equilibrium and the expression of B is detailed in Prop. 3.4.

From $\frac{dT^{(2)}}{dG} = -\left(\frac{\partial \Phi_T^{(1)}}{\partial G}\right)/\left(\frac{\partial \Phi_T^{(2)}}{\partial T}\right)$, from $B_{qs} < B_{eq}$, and from Eqs.(6.14,6.15,6.16), it follows that $\left(\frac{dT^{(2)}}{dG}\right)_{qs} > \left(\frac{dT^{(2)}}{dG}\right)_{eq}$. From $\frac{dF_2^{(2)}}{dG} = -\frac{dT^{(2)}}{dG}(R_T^{\text{Fin2}} - R_T^{\text{Oxi2}})/\chi_{F_2}^{tot}$ and Eq.(6.15) it follows that $\left|\frac{dF_2^{(2)}}{dG}\right|_{qs} > \left|\frac{dF_2^{(2)}}{dG}\right|_{eq}$. ∎

Proof of Proposition 4.5 We follows closely the proof of Prop. 4.2. The differences between $PPAR-/-$ and WT cells occur at two levels:

$$\left(R_{F_2}^{\text{Oxi1}}\right)_{PPAR-/-} = \left(R_{F_2}^{\text{Kout}}\right)_{PPAR-/-} = 0 \tag{6.17}$$

$$\left(R_{F_2}^{\text{Oxi2}}\right)_{WT,eq} > \left(R_{F_2}^{\text{Oxi2}}\right)_{PPAR-/-,eq}, \quad \left(\chi_{F_2}^{tot}\right)_{WT,eq} > \left(\chi_{F_2}^{tot}\right)_{PPAR-/-,eq},$$

$$\left(\rho_{F_2}^{\text{Oxi2}}\right)_{WT,eq} > \left(\rho_{F_2}^{\text{Oxi2}}\right)_{PPAR-/-,eq} \tag{6.18}$$

If $B_{WT,eq} > B_{PPAR-/-,eq}$, it follows (along the same lines as the proof of Prop. 4.2) that $\left(\frac{dT^{(2)}}{dG}\right)_{PPAR-/-,eq} > \left(\frac{dT^{(2)}}{dG}\right)_{WT,eq}$. From $\frac{dF_2^{(2)}}{dG} = -\frac{dT^{(2)}}{dG}(R_T^{\text{Fin2}} - R_T^{\text{Oxi2}})/\chi_{F_2}^{tot}$ and Eq.(6.18) it follows that $\left|\frac{dF_2^{(2)}}{dG}\right|_{eq,PPAR-/-} > \left|\frac{dF_2^{(2)}}{dG}\right|_{eq,WT}$. ∎

References

[ACW⁺03] K. Ashrafi, F.Y. Chang, J.L. Watts, A.G. Fraser, R.S. Kamath, J. Ahringer, and G. Ruvkun. Genome-wide rnai analysis of caenorhabditis elegans fat regulatory genes. *Nature*, 421:268–272, 2003.

[Bel05] J.M. Belovich. Modeling heterogeneity of liver metabolism. In *IUPS satellite meeting From Metabolome to Function Via Dynamic Measurements and Computational Models, San Diego*, 2005.

[BIBC79] RN Bergman, Y.Z. Ider, C.R. Bowden, and C. Cobelli. Quantitative estimation of insulin sensitivity. *Am J Physiol Endocrinol Metab Gastrointest Physiol*, 236:E667–E677, 1979.

[BLC⁺04] S. Barnouin, F. Lassere, M. Cantiello, H. Guillou, T. Pineau, and P. Martin. A kinetic view of coordinate modulations of gene expresion and metabolism in wild-type and pparα-/- mice during fasting. In *Conference of the Paul Hamel Institute, Monaco*, 2004.

[Cal05] H.B. Callen. *Thermodynamics and an introduction to thermostatics.* J.Wiley and Sons, 2005.

[Car05] D. Carling. Amp-activated protein kinase: balancing the scales. *Biochimie*, 87:87–91, 2005.

[CB95] A. Cornish-Bowden. *Fundamentals of Enzyme Kinetics.* Portland Press, 1995.

[CCRFS05] L. Calzone, N. Chabrier-Rivier, Fages F., and S. Soliman. A machine learning approach to biochemical reaction rules discovery. In *Proceedings of Foundations of Systems Biology in Engineering'05, Santa-Barbara*, 2005.

[CF03] K. Clément and P. Ferré. Genetics and the pathophysiology of obesity. *Pediatric Research*, 53:721–725, 2003.

[Cha02] C. et al. Chassagnole. Dynamic modeling of the central carbon metabolism of eschericia coli. *Biotechnology and Bioengineering*, 79:53–73, 2002.

[CRRT04] C. Chaouiya, E. Remy, P. Ruet, and D. Thieffry. Qualitative modelling of genetic networks: From logical regulatory graphs to standard petri nets. *Lecture Notes in Computer Science*, 3099:137–156, 2004.

[CWM05] L.D. Carsten, T. Watts, and T.A. Markow. Gene expression patterns accompanying a dietary shift in drosophila melanogaster. *Mol Ecology*, 14:3203–3208, 2005.

[CWS04] M. Castellanos, D.B. Wilson, and M.L. Shuler. A modular minimal cell model: Purine and pyrimidine transport and metabolism. *PNAS*, 101:6681–6686, 2004.

[DF04] E. Duplus and C. Forest. Is there a single mechanism for fatty acid regulation of gene transcription? *Biochemical Pharmacology*, 64:893–901, 2004.

[dJGH$^+$04] H. de Jong, J.-L. Gouzé, C. Hernandez, M. Page, T. Sari, and J. Geiselmann. Qualitative simulation of genetic regulatory networks using piecewise-linear models. *Bulletin of Mathematical Biology*, 66:301–340, 2004.

[ECB98] R. Eisenthal and A. Cornish-Bowden. Prospects for antiparasitic drugs: the case of *trypanasoma brucei*, the causative agent of african sleeping sickness. *J. Biol. Chem*, 272:5500–5505, 1998.

[Fel97] D. Fell. *Understanding the Control of Metabolism*. Portland Press, London, 1997.

[Fen79] N. Fenichel. Geometric singular perturbations theory for ordinary differential equations. *J.Diff.Eq.*, 31:53–98, 1979.

[Gou98] J.-L. Gouzé. Positive and negative circuits in dynamical systems. *J.Biol.Syst.*, 6:11–15, 1998.

[HS96] R. Heinrich and S. Schuster. *The Regulation of Cellular Systems*. Chapman and Hall, New York, 1996.

[Jum04] D.B. Jump. Fatty acid regulation of gene transcription. *Critical Rev. in Clinical Lab. Sci.*, 41:41–78, 2004.

[Kau93] S.A. Kauffman. *The origin of order, self-organisation and selection in evolution*. Oxford University Press, Oxford, U.K., 1993.

[La04] S. Lee and al. Requirement of pparα in maintaining phospolipid and triaglycerol homeostasis during energy deprivation. *J. Lipid Res.*, 4:2025–2037, 2004.

[LK02] M.J. Lambeth and M.J. Kushmerick. A computational model for glycogenolysis in skeletal muscle. *Annals of Biomedical Engineering*, 30:808–827, 2002.

[LSS$^+$05] P. Langley, O. Shiran, J. Shrager, L. Todorovski, and A. Pohorille. Constructing explanatory process models from biological data and knowledge. *AI in Medicine*, 2005.

[MDNM00] H. Matsuno, A. Doi, M. Nagasaki, and S. Miyano. Hybrid petri net representation of gene regulatory network. *Pac Symp Biocomput.*, 5:341–352, 2000.

[Men97] P. Mendes. Biochemistry by numbers: simulation of biochemical pathways with gepasi 3. *Trends Biochem. Sci.*, 22:36–363, 1997.

[MLR90] J-P. Mazat, T. Letellier, and C. Reder. Metabolic control theory: the geometry of the triangle. *Biomed Biochim Acta*, 49:801–810, 1990.

[MSBC06] P. Magni, G. Sparacino, R. Bellazzi, and C. Cobelli. Reduced sampling schedule for the glucose minimal model: importance of bayesian estimation. *Am J Physiol Endocrinol Metab*, 290:E177–E184, 2006.

[Mur03] J.D. Murray. *Mathematical Biology*, volume 1. Springer, 2003.

[Nob02] D. Noble. Modelling the heart: from genes to cells to the whole organ. *Science*, 295:1678–1682, 2002.

[Par83] T. Parthasarathy. *On Global Univalence Theorems*, volume 977 of *Lecture Notes in Mathematics*. Springer, 1983.

[PJM59] A.B. Perdee, F. Jacob, and J. Monod. The genetic control and cytoplasmatic expression of inducibility in the synthesis of β-galactosidase by e.coli. *J.Mol.Biol.*, 1:165–178, 1959.

[PLM03] J.-P. Pégorier and C. Le May. Régulation de l'expression génique par les acides gras control of gene expression by fatty acids. *Nutrition clinique et métabolisme*, 17:80–88, 2003.

[PRP$^+$03] ND Price, JL Reed, JA Papin, SJ Wiback, and Palsson BO. Network-based analysis of metabolic regulation in the human red blood cells. *J Theor Biol*, 225:185–194, 2003.

[PSP$^+$04] JA Papin, J Stelling, ND Price, S Klamt, S Schuster, and Palsson BO. Comparison of network-based pathway analysis methods. *Trends in Biotechnology*, 22:400–405, 2004.

[RLS$^+$06] O. Radulescu, S. Lagarrigue, A. Siegel, P. Veber, and M. Le Borgne. Topology and linear response of interaction networks in molecular biology. *Journal of The Royal Society Interface*, 3(6):185 – 196, 2006.

[Sal99] J.G. Salway. *Metabolism at a Glance*. Blackwell Publishers, 1999.

[Sno98] E.H. Snoussi. Necessary conditions for multistationarity and stable periodicity. *J.Biol.Syst.*, 6(3-9), 1998.

[Sou03] C. Soulé. Graphic requirements for multistationarity. *ComPlexUs*, 1:123–133, 2003.

[ST01] L. Sanchez and D. Thieffry. A logical analysis of the *drosophila* gap-gene system. *J. Theor. Biol.*, 211(115-141), 2001.

[TCB$^+$06] G. Toffolo, M. Campioni, R. Basu, R.A Rizza, and C. Cobelli. A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction. *Am J Physiol Endocrinol Metab*, 290:169–176, 2006.

[TCN03] John J. Tyson, C.K. Chen, and Béla Novák. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr. Opinion Cell Biol.*, 15:221–231, 2003.

[Tho81] R. Thomas. On the relation between the logical structure of systems and their ability to generate multiple steadt states or sustained oscillations. *Springer Ser. Synergetics*, 9:180–193, 1981.

[TPa00] B. Teusink, J. Passarge, and al. Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? testing biochemistry. *Eur J Biochem*, 267:5313–5329, 2000.

[TVS80] A. Tikhonov, A. Vasil'eva, and A. Sveshnikov. *Differential equations*. Springer, New York, 1980.

[VAS$^+$02] P. Vicini, A. Avogaro, M.E. Spilker, A. Gallo, and C. Cobelli. Quantitative estimation of insulin sensitivity. *Am J Physiol Endocrinol Metab*, 283:74–87, 2002.

[Was65] W. Wasov. *Asymptotic expansions for ordinary differential equations*. Interscience Publishers, 1965.

[YM03] N. Yildirim and M.C. Mackey. Feedback regulation in the lactose operon: a mathematical modeling study and comparison with experimental data. *Biophysical J.*, 84:2841–2851, 2003.