Genetically regulated metabolic networks: Gale-Nikaido modules and differential inequalities.

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Abstract. We propose an approach to study static properties of metabolic networks with genetic regulation. We base our results on differential inequalities which are constraints on the values of the partial derivatives of the reaction rate functions. The approach uses an iterative elimination method for the steady state equations involving algebraic modules that satisfy the Gale-Nikaido global univalence property. The same method allows to find conditions for unique steady state. In the case of metabolic pathways, partial elimination of variables can produce several alternative models, allowing to compare steady state changes of metabolites with and without genetic regulation.

Key words: systems biology, metabolic control, genetic regulation, qualitative constraints, univalence property

1 Introduction

The purpose of this paper is to illustrate a new methodology to analyze biological systems which lack numerical information and whose interactions can be structured according to function or timescales. We derive the conditions under which various static properties of these systems are satisfied and look for their biological interpretation. Our reasonings use information on the topology of the reaction network and on the way products regulate fluxes (inhibition or activation).

This work is motivated by the difficulty to build large quantitative models for physiological processes. Although complex biochemical models contain hundreds of reactions and chemical species, these models are relatively small compared to

models in combustion which contain thousands of reactions (see[1] for instance). The main reason that renders the study of biochemical networks difficult is not (as one may think) the number of variables, but the lack of information on kinetic parameters and, in many cases, the absence of complete knowledge of the mechanisms. It is thus important to develop symbolic tools for the study of biochemical networks, that do not use numerical information. As an example, given a model of genetically regulated metabolism we would like to know if the effect of a gene knock-out or of a change of the nutritional conditions will be an increase or a decrease in the value of a flux or of a concentration of a metabolite. Also, in order to understand the role of the genetic regulations to the stability and performance of metabolism, we would like to compare control coefficients of models with and without genetic regulations. Finally, we would like to find conditions for uniqueness of the steady state. All these motivations can be summarized by a single general question: how to constrain the sign of changes of steady states of perturbed biochemical models, mainly described by their topology and with no kinetic information?

Let us give more insight on methodological aspects. We derive differential models from the topology of the reaction network. Numeric or kinetic information is not required; this is supplied by a set of qualitative inequalities among derivatives of rate functions: roughly, we describe how reactions rates depend on each product of the system - increasing, decreasing, or independent. In order to analyze changes of steady states under genetic perturbation, we extend to a genetic framework the control coefficients introduced in metabolic control analysis [Fel97].

The main methodological problem we face is the computation of signs of control coefficients. To that matter, we introduce a formal method to relate fluxes and metabolites to inputs, based on the study of steady-state equations. Our method employs a powerful condition for uniqueness of steady states - the Gale-Nikaido theorem. We decompose the system into an increasing hierarchy of systems all having steady-states that match with the steady-states of the full system on the variables that are considered. In other words, steady-states of the full system are computed "step-by-step", allowing first the comparisons between the different steps and, second, the identification of those variables which may be implied in non-uniqueness. We use implicit function theorem to compute the hierarchy of control coefficients.

Our method may be considered as an improvement of two different fields. First, extensive studies have been performed on unique steady-state criteria based on the topology of the network [Tho81,CTF06]. Our method includes some of them, and provides new ones. Second, the field of predictions of reaction networks have been largely explored by flux balance analysis (FBA) [LGP06]. However, the results of such methodologies, depend on the judicious choice of the combination of fluxes to optimize. Furthermore, FBA predictions concern fluxes, not metabolites. The reduction methods and our extensions of metabolic

[1] http://kinetics.nist.gov/realfuels/

control allow us to extend the range of prediction, since we can discuss the effect of perturbations also on metabolites.

2 Formalism

2.1 Constraint based modeling

We consider chemical kinetic models defined as follows:

$$\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{p}) \qquad \mathbf{\Phi}(\mathbf{X}, \mathbf{p}) = \sum_{i}^{r} \nu_{\mathbf{i}} R_i(\mathbf{X}, \mathbf{p}),$$

where $\mathbf{X}$ denotes the concentration vector of products X_i; $\mathbf{p} \in \mathbb{R}^q$ stands for a set of parameters of the system; $\mathbf{\Phi} : \mathbb{R}^n \times \Delta \to \mathbb{R}^n$ where Δ is a compact subset of $\mathbb{R}^q$, is a differentiable vector field; $\nu_{\mathbf{i}}$ is the stoichiometric vector of the elementary reaction i; $R_i(\mathbf{X}, \mathbf{p})$ is the rate of elementary reaction i.

Our main assumption about the model will be that **The signs of the partial derivatives $\frac{\partial R_i}{\partial X_j}$ are constant and known**. This assumption is true for a very general class of systems, Michaelis-Menten, also power-law approximations for enzyme-catalyzed reactions, such as Generalized Mass Action and S-systems [SV87,KKS+06]. Although non-monotonic rates can be obtained by competitive regulatory mechanisms one could reasonably expect that more complex mechanisms can be decomposed into simple steps for which the constant sign condition is fulfilled. Alternatively, one may suppose that the constant sign condition is fulfilled in arcwise connected open domains of the phase space. For our study of static properties, namely steady state shifts, it will be enough to consider that both the initial and the final steady states are inside such a domain [RLS+06].

2.2 Steady states, sequences of box equilibration

A steady state is a solution of the system:

$$\mathcal{S} : \mathbf{\Phi}(\mathbf{X}, \mathbf{p}) = 0 \tag{2.1}$$

A partial steady state is a solution of $\mathbf{\Phi_1}(\mathbf{X_1}, \mathbf{X_2}, \mathbf{p}) = 0$ where $\mathbf{\Phi} = (\mathbf{\Phi_1}, \mathbf{\Phi_2})$, $\mathbf{X} = (\mathbf{X_1}, \mathbf{X_2})$ is an arbitrary splitting of the species in the model.

Our aim is to eliminate some or all variables in order to obtain properties of the system at steady state. We introduce a concept of box equilibration to perform the substitution method for non-linear systems of equations.

Box of a system of equations. We call box of the system (2.1) 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. A complete freedom is allowed to decide which variables are internal and which are external. This choice may of course be guided by biological reasons, but also by computational reasons. In this framework, internal variables are

those which are going to be removed from the systems. The elimination process described above ensures redistribution of regulatory effects of internal variables over the remaining part of the system. At first sight, the freedom given in the choice of internal variables is misleading since it may have no dynamical reason. However, this freedom shall be eventually considered as a strength since it allows us to focus on the steady states effects of any set variable of the system over other variable, abstracting from timescale and dynamical simulation viewpoints. As illustrated in the paper, this will allow us to understand better the effect of genetic regulation over the metabolic network, which was impossible with usual reduction methods based on dynamics since they prioritize long timescales (here genetic) to short timescales.

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 equilibration is the finite iteration of the following operations:

1. Define $\mathbf{X}_1 = \mathbf{X}$ and $\mathbf{\Phi_1}(\mathbf{X}_1, \mathbf{p}) = \mathbf{\Phi}(\mathbf{X}, \mathbf{p})$. We define $\mathcal{D}_1(\mathbf{p}) = \mathbb{R}^n_+$ as the maximal domain which contains solutions for the equation $\mathbf{\Phi_1}(\mathbf{X}_1, \mathbf{p}) = 0$.
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:
   - Let $\mathcal{D}_{k+1}(\mathbf{p})$ be the largest set of points $\mathbf{X_k^{(e)}}$ such that the equation $\mathbf{\Phi}_k^{(i)}(\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)}, \mathbf{p}) = 0$ has at least one solution $\mathbf{X}_k^{(i)}$, when $\mathbf{X_k^{(e)}}$ is considered as a fixed parameter, and such that $(\mathbf{X}_k^{(i)}, \mathbf{X}_k^{(e)}) \in \mathcal{D}_k(\mathbf{p})$. If $\mathcal{D}_{k+1}(\mathbf{p})$ is empty then stop: there is no solution. Thus, solving the equation allows expressing the internal variables as functions of the external variables $\mathbf{X}_k^{(i)} = \mathcal{M}_\mathbf{k}(\mathbf{X_k^{(e)}}, \mathbf{p})$. Notice that the solution might not be unique, that is $\mathcal{M}_\mathbf{k}$ is not necessarily univalent. We restrict our discussion to the case when the number of solutions is finite and bounded, such as for polynomial systems.
   - define $\mathbf{X_{k+1}} = \mathbf{X_k^{(e)}}$, and $\mathbf{\Phi_{k+1}} = \mathbf{\Phi_k^{(e)}}(\mathcal{M}_\mathbf{k}(\mathbf{X_n^{(e)}}, \mathbf{p}), \mathbf{X_n^{(e)}}, \mathbf{p})$.
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 equilibration 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 equilibration one should be able to express steady state species concentrations as functions of the external parameters: $\mathbf{X} = \mathcal{M}(\mathbf{p})$, where $\mathcal{M}$ results from a composition of the functions $\{\mathcal{M}_\mathbf{k}\}_{k=1,N_b}$:

$$\mathbf{X_k^{(i)}}(\mathbf{p}) = \mathcal{M}_\mathbf{k}(\mathbf{X_{k+1}^{(i)}}(\mathbf{p}), \ldots, \mathbf{X_{N_b}^{(i)}}(\mathbf{p})).$$

A well known example of (incomplete) sequence of box equilibration is a slow/fast reduction of a system: fast variables are reduced in order to obtain equivalent steady states for slow variables.

The sequence of equilibration is justified when the existence and uniqueness of solutions to the full system $\mathbf{\Phi} = 0$ are not straightforward. In the sequel, we will introduce conditions for existence and uniqueness. For computational (or biological significance) reasons, checking these conditions will be performed on subsets of variables and not on the full system itself. Hence, the concept of sequence of equilibration provides a flexibility in checking uniqueness conditions: we first equilibrate a set of internal variables satisfying uniqueness, perform reduction, then exhibit a new set of variables to be tested for uniqueness... At the end of the process, the set of variables that may be responsible for non-uniqueness of the steady state are eventually identified with this process. Additionally, performing reduction "step-by-step" allows us to derive biological interpretations of the conditions that would not be possible if the reduction would be performed in one step.

Existence and uniqueness of solutions. Box equilibration solves systems of equations by substitution. The existence and uniqueness of solutions relatively to box equilibration are straightforward.

Proposition 2.1. – *A solution of the system* (2.1) *exists for a value of the parameter p if there is a complete sequence of box equilibration with non-empty domains* $\mathcal{D}_{k+1}(\mathbf{p})$.
– *The function* $\mathcal{M}$ *is univalent (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 univalent on its maximal domain* $\mathcal{D}_{k+1}(\mathbf{p})$ *for a complete sequence of box equilibration.*

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 equilibration and to show that at each step the functions $\mathcal{M}_\mathbf{k}$ are univalent 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 equilibration we may still have an unique solution.

2.3 Checking box equilibration: existence of partial steady states

Let us state a sufficient condition for existence of steady states.

Theorem 2.1. *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 2.1 is based on the following standard mathematical lemma which is a consequence of the Poincaré-Hopf formula (see Additional material in the Appendix).

Lemma 2.1. *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.

Biological interpretation. This theorem is very general but it can be considered quite easily in a biological setting: $\mathbf{G}$ may be considered as the result of production and consumption of products by biochemical reactions with a saturation effect, whereas $\mathbf{\Lambda}$ represents the (unbounded) product degradation. More precisely, the hypotheses of Theorem 2.1 are fulfilled by rather general networks of biochemical reactions, by assuming the following rules:

- For each variable X_i, degradation terms $\mathrm{Dg}(X_i)$ are increasing function of X_i with no saturation effect.
- In the absence of substrates all fluxes vanish.
- All fluxes except degradation saturate at high concentrations of metabolites, implying that production terms $\mathbf{\Phi}$ are bounded.
- There exists a recovery effect on each metabolic variable. By recovery effect we mean that if a variable is zero, then at least one reaction that produces the variable is active.

The following consequence of Theorem 2.1 is very useful to exhibit complete sequences of box equilibration.

Corollary 2.1. *Let* $\mathbf{X} = (\mathbf{X_1}, \mathbf{X_2})$, $\Phi = (\Phi_1, \Phi_2)$ *be any partition of the variables. We suppose that* $\Phi_1(\mathbf{X_1}, \mathbf{X_2})$ *satisfies the hypotheses of Theorem 2.1, as a function of* $\mathbf{X_1}$. *Then, 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.*

2.4 Checking box equilibration: uniqueness of partial steady state

In order to identify boxes of equilibration, we introduce an algebraic sufficient condition on signs of derivatives for uniqueness. 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 $M = (m_{i,j})_{i,j \in \{1,\ldots,n\}}$ is defined as $\Delta_I = \det M_I$, where $I \subset \{1, \ldots, n\}$ and $M_I = (m_{i,j})_{i,j \in I}$.

Theorem 2.2 (Gale-Nikaido). *If $\mathbf{X} \to \mathbf{\Phi}(\mathbf{X})$ is a differentiable mapping from $\mathbb{R}^n_+$ to $\mathbb{R}^n$, of Jacobian J, such that all the principal minors of $-J$ are positive, then this mapping is globally univalent. In particular the system $\mathbf{\Phi} = 0$ has a unique solution if a solution exists.*

Recovering Thomas condition for uniqueness of steady states.

Let us detail why this result can be seen as a generalization of the well known Thomas condition for uniqueness of the steady state. The interaction graph is the signed, oriented graph on the variables derived from the Jacobian J of the model: j is connected to i ($j \to i$) iff $J_{ij} \neq 0$. The sign of the connecting arc is the sign of J_{ij}. Many known topological conditions for uniqueness of steady state actually follow from the cycle decomposition of the determinant of $-J$, that is, $\Delta(-J) = \sum_{L \in \mathcal{L}} (-1)^{|L|} lp(L)$, where $\mathcal{L}$ is the set of all cycle partitions and $|L|$ is the number of cycles in the partition L, $lp(L)$ is the product of elements J_{ij} for all the arcs in L. As a particular case, assume that there are no positive cycles in the interaction graph (Thomas sufficient condition for uniqueness of steady state [Tho81]). Consider a principal minor J and the corresponding subgraph $\mathcal{G}$. Let L be a partition of $\mathcal{G}$ into $|L|$ disjoint cycles $l_1 \dots l_{|L|}$. Each cycle l_k is also a cycle of the full interaction graph, implying that the product of signs in each cycle l_k is negative. Therefore, the sign $lp(L)$ of arcs in the partition L equals $(-1)^{|L|}$. Summing up these relation yields $\Delta(-J) = \sum_{L \in \mathcal{L}} (-1)^{|L|} lp(L) = \sum_{L \in \mathcal{L}} (-1)^{2|L|} = |\mathcal{L}| > 0$. From Theorem 2.2 we get the uniqueness of the steady state.

Limitations of Thomas conditions Nevertheless, Thomas condition is too restrictive for most of the applications, especially to metabolism. Indeed, a reversible reaction can be represented as a set of two reactions of opposite stoichiometry: $X_i \to X_j$ and $X_j \to X_i$ of rates $R(X_i, X_j)$, $R'(X_j, X_i)$, respectively. Since X_i, X_j are substrates of R, R', we have $\frac{\partial R}{\partial X_i} > 0$ and $\frac{\partial R'}{\partial X_j} > 0$ and it follows that $i \to j \to i$ is a positive loop in the interaction graph; Thomas condition does not apply.

Let us go further in this example. We notice that contribution of reactions R, R' to the decomposition of $\Delta(-J)$ is zero, because the contribution to any cycle partition containing $i \to j \to i$ is exactly canceled by the contribution to the cycle partition containing $i \to i$ and $j \to j$. Actually, checking formally the Gale-Nikaido condition allows us to avoid the limitations of the Thomas condition.

A reversible reaction corresponds to a non-essential loop in the interaction graph, i.e. a loop not contributing to multi-stationarity. In this paper we check uniqueness of steady state by direct application of the Gale-Nikaido condition. Non-essential loops have vanishing contribution to principal minors of the Jacobian and are thus automatically eliminated by this procedure.

3 Constraint based model for fatty acids metabolism

We apply our formalism to a minimal mixed metabolic and genetic model of regulated fatty acids metabolism in liver. To set ideas, all the variables of the model pertain to an "abstract" hepatocyte, capable of the two different functioning modes. We thus voluntarily reduced the set of elements in the model. These belongs to three different classes of molecules. Their corresponding symbols are given in Table 3.1.

- **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.
- **Metabolic variables**: Acetyl-CoA is the first brick for building fatty acids; Saturated and monounsaturated fatty acids (denoted by S/MU-FA) are produced either by the organisms from Acetyl-CoA or brought by the diet; Exogenous polyunsaturated fatty acids (PUFA) are entering the metabolism only as part of the diet.
- **Energetic variable**: a variable ATP expresses the energy that the cell has at its disposal.
- **Genetic variables**: we introduce abstract enzymes for each set of enzymes that are involved in a metabolic pathway and main transcription factors known to regulate these enzymes, namely, the active form of the nuclear receptor PPAR and the active form of the nuclear receptor LXR, representing in a very simplified way the regulation path LXRα-SREBP-1.

In different species such as poultry, rodents and humans, hepatocyte (liver) cells have the specificity to ensure both lipogenesis and β-oxidation. We thus abstracted the main fluxes and regulations implied in this biological process.

- **Metabolic fluxes**. Glycolysis produces Acetyl-CoA from glucose. Krebs cycle produces energy for cellular needs from Acetyl-CoA. Ketone bodies exit allows the cell to transfer the energy stored in Acetyl-CoA to the outside, allowing survival during fasting. Lipogenesis transforms Acetyl-CoA into S/MU-FA via citrate. Then an outtake flux allows S/MU-FA to exit liver and go to storing tissues (adipocytes); this flux is reversible since the intake flux is fed partially from diet, partially from lipolysed adipocytes. Additionally, the intake/outtake flux of PUFA allows PUFA to enter or exit the cell, including a synthetic pathway consisting of desaturation and elongation of essential fatty acids. When fatty acids enter the cell, a $\beta-$oxidation burns all fatty acids in order to produce energy and to recover Acetyl-CoA. Finally, ATP consumption expresses the energy (ATP) the cell consumes for living. Degradation of metabolites can not be neglected on the genetic timescale.
- **Genetic regulations.** Fluxes are regulated by their sets of enzymes, which are themselves regulated by transcription factors PPARα and LXRα. More precisely, LXR and SREBP-1 triggers S/MU-FA synthesis enzymes production and PPAR triggers the production of S/MU-FA oxydation enzymes, PUFA oxydation enzymes and ketone exit enzymes.

- **Activity regulations.** 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 (see details in [CF03,PLM03,DF04,Jum04]). Although the precise mechanisms have not been proved yet, some well established facts are used for modeling: PUFA increases PPAR activity and inhibits LXR activity.
- **Energetic regulations on fat intake**. Fat intake is needed to produce energy by oxidation. A drop in energy (ATP) stimulates fat intake.

Our full model for fatty acid metabolism and its regulations is depicted in Table 3.1. It was built from these interactions by using the following rules.

- 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 stoichiometry. Thus, the fluxes Gly, Krb, Ox1, Ox2, Sy are considered to have the stoichiometry's $\mathrm{G} \to \mathrm{A}+\alpha_G T$, $\mathrm{A} \to \alpha_K T$, $\mathrm{F}_1 \to n_1\mathrm{A}+\alpha_{O1} T$, $\mathrm{F}_2 \to n_1\mathrm{A}+\alpha_{O2} T$, $n_1\mathrm{A} + \alpha_S T \to \mathrm{F}_1$ respectively.
- Degradation reactions of metabolites are supposed to be linear: $\mathrm{DgV(V)} = \delta_{\mathrm{V}}\mathrm{V}$ 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.

The information already given about regulations allows to partially fill the table of partial derivatives of the fluxes. To identify the remaining signs, we use the following assumptions: a)Substrate effect, an increase of substrate increases the associated flux; b) Transport effects, intake/outtake fluxes In1 and In2 are conventionally directed to the inside, they decrease when the internal concentrations of fatty acids increase; c) Product negative feed-back, fluxes producing ATP are negatively controlled by ATP. The resulting table of derivation is given in Table 3.2.

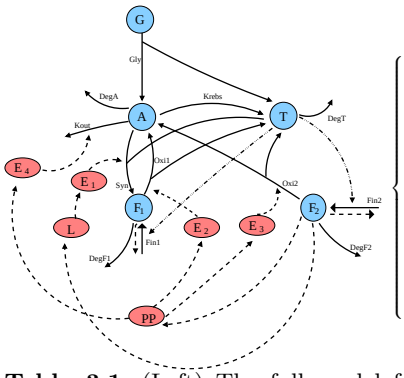
4 Results

We apply the results detailed in the first section to derive several information about fatty acid metabolism.

4.1 Two models of response of the metabolism

Genetically non-regulated model. A genetically regulated system is multi-scale. During fast response genetic variables can be considered to be constant and equal to their initial values. We call genetically non-regulated model the reduced model obtained from the full model by considering $E_i(t) = E_i(0), i = 1, \ldots, 4$, $\mathrm{PP}(t) = \mathrm{PP}(0)$, $\mathrm{L}(t) = \mathrm{L}(0)$. Fast response is obtained at partial steady

Type	Name	Concentration symbol	$\frac{d\,\text{product}}{dt}$
Metabolic parameter	Glucose	G	
Metabolic variable	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}
Energetic variable	Energy ATP	T	Φ_{T}
Genetic variable	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



$$
\begin{cases}
\frac{d\mathrm{A}}{dt} &= \mathrm{Gly}(\mathrm{G},\mathrm{T}) + n_1\mathrm{Ox1}(\mathrm{F}_1,\mathrm{T},\mathrm{E}_2) + n_2\mathrm{Ox2}(\mathrm{F}_2,\mathrm{T},\mathrm{E}_3) \\
&\quad -\mathrm{Krb}(\mathrm{A},\mathrm{T}) - \mathrm{Ko}(\mathrm{A},\mathrm{E}_4) - n_1\mathrm{Sy}(\mathrm{A},\mathrm{T},\mathrm{E}_1) - \delta_{\mathrm{A}}\mathrm{A} \\
\frac{d\mathrm{F}_1}{dt} &= \mathrm{Sy}(\mathrm{A},\mathrm{T},\mathrm{E}_1) - \mathrm{Ox1}(\mathrm{F}_1,\mathrm{T},\mathrm{E}_2) + \mathrm{In1}(\mathrm{F}_1,\mathrm{T}) - \delta_{\mathrm{F}_1}\mathrm{F}_1 \\
\frac{d\mathrm{F}_2}{dt} &= -\mathrm{Ox2}(\mathrm{F}_2,\mathrm{T},\mathrm{E}_3) + \mathrm{In2}(\mathrm{F}_2,\mathrm{T}) - \delta_{\mathrm{F}_2}\mathrm{F}_2 \\
\frac{d\mathrm{T}}{dt} &= \alpha_{\mathrm{G}}\mathrm{Gly}(\mathrm{G},\mathrm{T}) + \alpha_{\mathrm{K}}\mathrm{Krb}(\mathrm{A},\mathrm{T}) + \alpha_{\mathrm{O1}}\mathrm{Ox1}(\mathrm{F}_1,\mathrm{T},\mathrm{E}_2) \\
&\quad +\alpha_{\mathrm{O2}}\mathrm{Ox2}(\mathrm{F}_2,\mathrm{T},\mathrm{E}_3) - \alpha_S\mathrm{Sy}(\mathrm{A},\mathrm{T},\mathrm{E}_1) - \mathrm{DgT}(\mathrm{T}) \\
\frac{d\mathrm{PP}}{dt} &= \tilde{\Psi}_1(\mathrm{F}_2) - \delta_{\mathrm{PP}}\mathrm{PP} \\
\frac{d\mathrm{L}}{dt} &= \tilde{\Psi}_2(\mathrm{F}_2) - \delta_{\mathrm{L}}\mathrm{L} \\
\frac{d\mathrm{E}_1}{dt} &= \tilde{\Psi}_3(\mathrm{L}) - \delta_{\mathrm{E}_1}\mathrm{E}_1 \\
\frac{d\mathrm{E}_2}{dt} &= \tilde{\Psi}_4(\mathrm{PP}) - \delta_{\mathrm{E}_2}\mathrm{E}_2 \\
\frac{d\mathrm{E}_3}{dt} &= \tilde{\Psi}_5(\mathrm{PP}) - \delta_{\mathrm{E}_3}\mathrm{E}_3 \\
\frac{d\mathrm{E}_4}{dt} &= \tilde{\Psi}_6(\mathrm{PP}) - \delta_{\mathrm{E}_4}\mathrm{E}_4
\end{cases}
$$

Table 3.1. (Left) The full model for fatty acid metabolism. Dashed arrows stand for genetic actions from the origin on to target. Plain arrows stand for metabolic fluxes. Dash-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).
(Right) Differential equations for the full model. The flux of each metabolic variable is obtained as a mass balance of primitive fluxes.

$\frac{\partial\,\text{flux}}{\partial\,\text{var.}}$	Gly	Krb	Ko	Sy	Ox1	Ox2	In1	In2	DgT	$\tilde{\Psi}_1$	$\tilde{\Psi}_2$	$\tilde{\Psi}_3$	$\tilde{\Psi}_4$	$\tilde{\Psi}_5$	$\tilde{\Psi}_6$
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 3.2. Constrained signs on the full model for regulated fatty acid metabolism in liver.

state of the remaining four variables $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2, \mathrm{T}\}$. By construction, the genetically non-regulated model can be used to describe the rapid response of the metabolic variables on timescales smaller than the relaxation time of the genetic variables. In particular, the steady state of this model are the quasi-stationary states of the full model. **The genetically regulated model.** Slow adiabatic response involves all variables, including genetic ones. Nonetheless, the static response of the system can be obtained by arbitrarily choosing the order of partial equilibration. In order to study the impact of genetic regulation on energetic homeostasis, we consider a second reduced model involving the same four variables. In the full model the box formed by the species $\{E_1, E_2, E_3, E_4, \mathrm{PP}, \mathrm{L}\}$ is acyclic and satisfies the conditions of Corollary 2.1, thus partial steady state exists and is unique. We call genetically regulated model the reduction of the full model to the set $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2, \mathrm{T}\}$ (obtained by elimination of the genetic variables). The chain rule formula allows to calculate the signs of flux derivatives obtained after reduction. The genetically regulated model and the full model do not simulate the same dynamics, but their steady states are identical over the set of variables $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2, \mathrm{T}\}$.

The genetically regulated model and the genetically non-regulated model have the same structure in terms of variables, parameters and fluxes. However, performing reductions affects the dependencies of genetically regulated fluxes Ko, Sy, Ox1, Ox2, In1, In2 on other variables. The value of these fluxes after elimination is denoted by subscripts gr (standing for genetically regulated) or *gnr* (for non genetically regulated), for instance $\mathrm{Ko}_{\mathrm{gr}}$ or Ko_{gnr}. The models are detailed in Table 4.1 together with the corresponding sign table. As shown in the table, the only difference is in the regulation: some fluxes do not depend on F_2 in the genetically non-regulated model, since the regulations of F_2 are different in the genetically non-regulated and genetically regulated models, leading to two different functions F_2^{gnr} and $\mathrm{F}_2^{\mathrm{gr}}$.

Notice that the two models models do not simulate the same dynamics and that their steady states match, on the metabolic variables, with the value of quasi-stationary and steady states of the full model, respectively. Comparing the steady states of these two models will allow a characterization of the effect of genetic regulations on the full model.

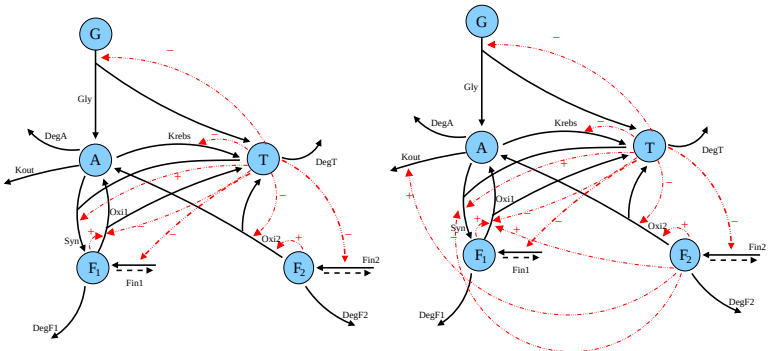
Control coefficients and elasticities. We call control coefficients the derivatives of fluxes with respect to F_2 and T. We also call elasticity the derivative of the logarithm of the rate of metabolic variables with respect to the logarithm of the substrate concentration: they quantify how rates and fluxes of a metabolite depend on this metabolite [CB95]. Corresponding symbols are given in Table 4.1. These quantities are defined such that they are all positive.

4.2 Condition for unique steady state

We can now turn to the application of our theoretical results about existence and uniqueness of equilibria. First, let us notice that hypotheses of Theorem 2.1

$$
\begin{cases}
\frac{dA}{dt} &= -\delta_A A + \mathrm{Gly}(G,T) + n_1 \mathrm{Ox1}_{gr,gnr}(F_1,F_2,T) + n_2 \mathrm{Ox2}_{gr,gnr}(F_2,T) - \mathrm{Krb}(A,T) - \\
&\quad \mathrm{Ko}_{gr,gnr}(A,F_2) - n_1 \mathrm{Sy}_{gr,gnr}(A,F_2,T) \\
\frac{dF_1}{dt} &= \mathrm{Sy}_{gr,gnr}(A,F_2,T) - \mathrm{Ox1}_{gr,gnr}(F_1,F_2,T) + \mathrm{In1}(F_1,T) - \delta_{F_1} F_1 \\
\frac{dF_2}{dt} &= -\mathrm{Ox2}_{gr,gnr}(F_2,T) + \mathrm{In2}(F_2,T) - \delta_{F_2} F_2 \\
\frac{dT}{dt} &= \alpha_K \mathrm{Krb}(A,T) + \alpha_{O1} \mathrm{Ox1}_{gr,gnr}(F_1,F_2,T) + \alpha_{O2} \mathrm{Ox2}_{gr,gnr}(F_2,T) - \alpha_S \mathrm{Sy}_{gr,gnr}(A,F_2,T) \\
&\quad + \alpha_G \mathrm{Gly}(G,T) - \mathrm{DgT}(T)
\end{cases}
$$

$\frac{\partial \Phi}{\partial X}$	Gly	Krb	Ko	Sy	Ox1	Ox2	In1	In2	DgT
A	0	χ_A^{Krb}	+	χ_A^{Sy}	0	0	0	0	0
F_1	0	0	0	0	$\chi_{F_1}^{Ox1}$	0	−	0	0
F_2 $^{gnr}_{gr}$	0	0	$\begin{matrix}0\\ R_{F_2}^{Ko}\end{matrix}$	$\begin{matrix}0\\ -R_{F_2}^{Sy}\end{matrix}$	$\begin{matrix}0\\ R_{F_2}^{Ox1}\end{matrix}$	$R_{F_2}^{Ox2}$	0	−	0
T	$-R_T^{Gly}$	$-R_T^{Krb}$	0	R_T^{Sy}	$-R_T^{Ox1}$	$-R_T^{Ox2}$	$-R_T^{In1}$	$-R_T^{In2}$	+
G	+	0	0	0	0	0	0	0	0



a) genetically non-regulated model. b) genetically regulated model.

Table 4.1. The genetically non-regulated and genetically regulated models given as differential equations and graph. The differential equations simulate the correct dynamics only for the genetically non-regulated model, and only on the rapid part of the trajectory before reaching quasi-stationarity. For genetically regulated model they only provide the same steady state as the full model, a condition which is sufficient for the study of static properties. The table of signs contains symbols corresponding to control coefficients and elasticities. Notations *gnr* and gr denote the value of fluxes for the different models (non-genetically or genetically regulated). All the symbols stand for positive values. These quantities inform on the strength of fluxes variations one with respect to the other and how rates and fluxes of a metabolite depend on this metabolite. They will be used in the sequel to express conditions on the system to satisfying specific behaviors.

apply to the full model and to the reduced models. All these models admit at least a steady state.

Bistability of genetically regulated metabolism is used by some organisms to adapt to a change in food (see the operon lactose in E.coli). 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. This motivates the first biological question we wish to answer: in higher organisms, does the whole of regulations produce bistability or a unique steady state of fatty acid metabolism, or is the change from lipogenesis to lipolysis merely steady state shift?

In order to answer this question, we will use the Gale-Nikaido Theorem 2.2 to exhibit a complete sequence of univalent equilibration: the first box is $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2\}$ and the second box $\{\mathrm{T}\}$. This will allow us to exhibit an algebraic condition for unique steady state. The biological relevance of this condition will be discussed at the end of the section.

To this matter, we introduce new elasticities, all positive:

$$\chi^{tot}_{\mathrm{F}_1} = -\frac{\partial \Phi_{\mathrm{F}_1}}{\partial \mathrm{F}_1}, \quad \chi^{tot}_{\mathrm{F}_2} = -\frac{\partial \Phi_{\mathrm{F}_2}}{\partial \mathrm{F}_2}, \quad \chi^{tot}_{\mathrm{A}} = -\frac{\partial \Phi_{\mathrm{A}}}{\partial \mathrm{A}}.$$

We can also show that the following ratios are all positive and strictly less than 1:

$$\rho^{\mathrm{Ox1}}_{\mathrm{F}_1} = \frac{\chi^{\mathrm{Ox1}}_{\mathrm{F}_1}}{\chi^{tot}_{\mathrm{F}_1}}, \ \rho^{\mathrm{Sy}}_{\mathrm{A}} = \frac{n_1 \chi^{\mathrm{Sy}}_{\mathrm{A}}}{\chi^{tot}_{\mathrm{A}}}, \ \rho^{\mathrm{Ox2}}_{\mathrm{F}_2} = \frac{R^{\mathrm{Ox2}}_{\mathrm{F}_2}}{\chi^{tot}_{\mathrm{F}_2}}$$

Let us define the following combinations of control coefficients and elasticities.

$$\begin{aligned}
A &= X\rho^{\mathrm{Ox1}}_{\mathrm{F}_1}, \qquad X = n_1(\alpha_{\mathrm{O1}} - \alpha_S \rho^{\mathrm{Sy}}_{\mathrm{A}} + n_1 \alpha_K \rho^{Krebs}_{A}),\\
B &= B_1 R^{\mathrm{Sy}}_{\mathrm{F}_2}/\chi^{tot}_{\mathrm{F}_2} + B_2 R^{\mathrm{Ko}}_{\mathrm{F}_2}/\chi^{tot}_{\mathrm{F}_2} + B_3 R^{\mathrm{O1}}_{\mathrm{F}_2}/\chi^{tot}_{\mathrm{F}_2} + B_4 \rho^{\mathrm{Ox2}}_{\mathrm{F}_2},\\
B_1 &= X - n_1(\alpha_{\mathrm{O1}} - \alpha_S)(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}} \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}), \qquad B_2 = \alpha_{\mathrm{O1}}(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}} \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) - X/n_1,\\
B_3 &= X(1 - \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}), \quad B_4 = n_1 \alpha_{\mathrm{O2}}(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}} \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) + n_2/n_1 X - n_2 \alpha_{\mathrm{O1}}(1 - (\rho^{\mathrm{Sy}}_{\mathrm{A}})^2 \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}),\\
C &= [X/n_1 + (n_1\alpha_G - \alpha_{\mathrm{O1}})(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}} \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) R^{Gly}_T + [\alpha_{\mathrm{O1}} + n_1 \alpha_K + X R^{Oxi1}_T \qquad (4.1)\\
&\quad [n_2/n_1 X + n_2 \alpha_S \rho^{\mathrm{Sy}}_{\mathrm{A}}(1 - \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) + (n_1\alpha_{\mathrm{O2}} - n_2\alpha_{\mathrm{O1}})(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}} \rho^{\mathrm{Ox1}}_{\mathrm{F}_1})] R^{Oxi2}_T +\\
&\quad -(\alpha_S + X/n_1)\rho^{\mathrm{Ox1}}_{\mathrm{F}_1} + \rho^{\mathrm{Sy}}_{\mathrm{A}}] R^{Krebs}_T + [n_1(\alpha_S - \alpha_{\mathrm{O1}}) + X](1 - \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) R^{Syn}_T,\\
D &= [X/n_1 - \alpha_{\mathrm{O1}}(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}})] R^{Krebs}_T + n_2 \alpha_S \rho^{\mathrm{Sy}}_{\mathrm{A}}(1 - \rho^{\mathrm{Ox1}}_{\mathrm{F}_1}) R^{Oxi2}_T\\
&\quad + n_1(\alpha_{\mathrm{O1}} - \alpha_S)(1 - \rho^{\mathrm{Sy}}_{\mathrm{A}}) \rho^{\mathrm{Ox1}}_{\mathrm{F}_1} R^{S}_T.
\end{aligned}$$

As detailed in the proof of Theorem 4.1 above, theses combinations result from box equilibration – Section 2.2 – together with the Gale-Nikaido uniqueness condition – Theorem 2.2 – and the implicit function theorem. All together, we obtain combinations of coefficients that compose the steady-state uniqueness conditions for the models we are considering. The biological interpretation of these coefficients is discussed at the end of the present section.

Theorem 4.1. *Assume that the following strong lipolytic response condition (4.2) and fatty acid proportion condition (4.3) are fulfilled at fixed genetic variables and at genetic partial steady state, for every* $\mathrm{G} \in [0, G_{max}]$. *Suppose additionally that that the stoichiometry condition (4.4) is satisfied:*

$$A(R^{\mathrm{In1}}_{\mathrm{T}} - R^{\mathrm{Ox1}}_{\mathrm{T}}) + C > D, \tag{4.2}$$

$$|B(R^{\mathrm{In2}}_{\mathrm{T}} - R^{\mathrm{Ox2}}_{\mathrm{T}})| <<< A|R^{\mathrm{In1}}_{\mathrm{T}} - R^{\mathrm{Ox1}}_{\mathrm{T}}| \tag{4.3}$$

$$\alpha_S < \alpha_{\mathrm{O1}} < n_1 \alpha_G, \qquad n_2 \alpha_{\mathrm{O1}} < n_1 \alpha_{\mathrm{O2}}. \tag{4.4}$$

Then the model of fatty acid metabolism has a unique steady state, with or without genetic regulation. The quantities A, C, D are positive.

Proof. Let us first prove that for all $(\mathrm{G},\mathrm{T}) \in [0, G_{max}] \times \mathbb{R}_+$, the box $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2\}$ can be eliminated from equilibria equations in the genetically non-regulated model and the genetically regulated model. Corollary 2.1 implies that the system of equations $\Phi_{\mathrm{A}}(\mathrm{G},\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T}) = \Phi_{\mathrm{F}_1}(\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T}) = \Phi_{\mathrm{F}_2}(\mathrm{F}_2,\mathrm{T}) = 0$ has a solution for every fixed (G,T). To prove the uniqueness of the solution to the system, we apply Theorem 2.2 to the mapping $(\mathrm{A},\mathrm{F}_1,\mathrm{F}_2) \to (\Phi_{\mathrm{A}}, \Phi_{\mathrm{F}_1}, \Phi_{\mathrm{F}_2})$; let $J^{(1)}$ is the Jacobian of this mapping:

$$J^{(1)} = \begin{pmatrix} -\chi_{\mathrm{A}}^{tot} & n_1\chi_{\mathrm{F}_1}^{\mathrm{Ox1}} & n_2 R_{\mathrm{F}_2}^{\mathrm{Ox2}} + n_1 R_{\mathrm{F}_2}^{\mathrm{Ox1}} + n_1 R_{\mathrm{F}_2}^{\mathrm{Sy}} - R_{\mathrm{F}_2}^{\mathrm{Ko}} \\ \chi_{\mathrm{A}}^{\mathrm{Sy}} & -\chi_{\mathrm{F}_1}^{tot} & -R_{\mathrm{F}_2}^{\mathrm{Ox1}} - R_{\mathrm{F}_2}^{\mathrm{Sy}} \\ 0 & 0 & -\chi_{\mathrm{F}_2}^{tot} \end{pmatrix}.$$

We ensure that all the principal minors of $-J^{(1)}$ are all positive: $\chi_{\mathrm{A}}^{tot} > 0$, $\chi_{\mathrm{A}}^{tot}\chi_{\mathrm{F}_1}^{tot} - n_1\chi_{\mathrm{A}}^{\mathrm{Sy}}\chi_{\mathrm{F}_1}^{\mathrm{Ox1}} = \chi_{\mathrm{A}}^{tot}\chi_{\mathrm{F}_1}^{tot}(1-\rho_{\mathrm{A}}^{\mathrm{Sy}}\rho_{\mathrm{F}_1}^{\mathrm{Ox1}}) > 0$, $\chi_{\mathrm{F}_2}^{tot}\chi_{\mathrm{A}}^{tot}\chi_{\mathrm{F}_1}^{tot}(1-\rho_{\mathrm{A}}^{\mathrm{Sy}}\rho_{\mathrm{F}_1}^{\mathrm{Ox1}}) > 0$, as a consequence of the sign table. This is valid both at fixed genetic variables and at partial steady state of genetic variables.
Alternatively this can be seen from the topology of the reaction network. The only cycle of the box $\{\mathrm{A}, \mathrm{F}_1, \mathrm{F}_2\}$ comes from the pair of opposed fluxes Sy, Ox1; or, these are hanging equations.
Since Theorem 2.2 applies, there exist functions $\mathrm{A}^{(1)}(\mathrm{G},\mathrm{T})$, ${\mathrm{F}_1}^{(1)}(\mathrm{G},\mathrm{T})$ and ${\mathrm{F}_2}^{(1)}(\mathrm{G},\mathrm{T})$ that are the unique solutions of the system $\Phi_{\mathrm{A}}(\mathrm{G},\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T}) = \Phi_{\mathrm{F}_1}(\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T}) = \Phi_{\mathrm{F}_2}(\mathrm{F}_2,\mathrm{T}) = 0$ for each (G,T). These functions are differentiable on $\mathbb{R}_+^2$ by the implicit function theorem.

We then introduce ${\Phi_{\mathrm{T}}}^{(1)}(\mathrm{G},\mathrm{T}) = \Phi_{\mathrm{T}}(\mathrm{G},\mathrm{A}^{(1)}(\mathrm{G},\mathrm{T}),{\mathrm{F}_1}^{(1)}(\mathrm{G},\mathrm{T}),{\mathrm{F}_2}^{(1)}(\mathrm{G},\mathrm{T}),\mathrm{T})$. The biological hypotheses imply that Theorem 2.1 applies and unicity in equations for A, F_1, F_2 implies that the function ${\Phi_{\mathrm{T}}}^{(1)}(\mathrm{G},T)$ has a root in T for every G. We deduce that a sufficient condition for unicity is given by the Gale-Nikaido theorem applied on the model reduced to the variable G; more precisely, the function ${\Phi_{\mathrm{T}}}^{(1)}$ is differentiable on $\mathbb{R}_+^2$. From the definition of the function ${\Phi_{\mathrm{T}}}^{(1)}$ it follows $\frac{\partial {\Phi_{\mathrm{T}}}^{(1)}}{\partial \mathrm{T}} = \frac{\partial \Phi_{\mathrm{T}}}{\partial \mathrm{T}} + (\alpha_{\mathrm{K}}\chi_{\mathrm{A}}^{\mathrm{Krb}} - \alpha_S\chi_{\mathrm{A}}^{\mathrm{Sy}})\frac{\partial \mathrm{A}^{(1)}}{\partial \mathrm{T}} + \alpha_{\mathrm{O1}}\chi_{\mathrm{F}_1}^{\mathrm{Ox1}}\frac{\partial {\mathrm{F}_1}^{(1)}}{\partial \mathrm{T}} + (\alpha_{\mathrm{O1}}R_{\mathrm{F}_2}^{\mathrm{Ox1}} + \alpha_{\mathrm{O2}}R_{\mathrm{F}_2}^{\mathrm{Ox2}} + \alpha_S R_{\mathrm{F}_2}^{\mathrm{Sy}})\frac{\partial {\mathrm{F}_2}^{(1)}}{\partial \mathrm{T}}$.
We show by formal manipulation by using Mathematica version 5.2 software (in the derivation we neglect terms involving F_2, because of (4.3)) that the strong lipolytic conditions implies $\frac{\partial {\Phi_{\mathrm{T}}}^{(1)}}{\partial \mathrm{T}} < 0$. In other words, ${\Phi_{\mathrm{T}}}^{(1)}$ is monotonic so that ${\Phi_{\mathrm{T}}}^{(1)}(\mathrm{G},\mathrm{T})$ has a unique zero for every G. Let $T = \mathrm{T}^{(2)}(\mathrm{G})$ be the solution of this equation. ∎

Biological interpretation. We now turn to the interpretation of the algebraic conditions. Although the systems of conditions has been reduced to a very abstract and condensed shape, exhibiting numerical coefficients to prove that the conditions are satisfied is not always possible. In order to do that we need either a (at least partially) parametrized model from the very beginning, or a series of experiments to estimate the control coefficients. As an alternative, let us express

the algebraic conditions introduced in Theorem 4.1 as biological conditions over the relative strengths of fluxes and their dependencies on the products of the system.

- The stoichiometry condition (4.4) can be checked from biochemical data, by considering the average numbers of Acetyl-coA and ATP molecules produced or consumed by the different fluxes.
- The strong lipolytic condition (4.2) means that the energy variation has a sufficiently strong effect on the arrival of fatty acids inside the cell. 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. This suggests that the strong lipolytic condition is satisfied. To certify that the condition is always satisfied, we would nevertheless need experiments for every T constrained states as well.
- The fatty acid proportion condition (4.3) means that polyunsaturated fatty acids are minority among all FA, which is stated for instance in [La04].

It follows from this discussion that the conditions of uniqueness are reasonable for the biological viewpoint, which suggests that fatty acid metabolism and their regulation correspond to a model with a unique steady state instead of a bistable one.

4.3 Predictions of the model and some validations

(a) Role of genetic regulations in energy homeostasis Let $T = \mathrm{T}^{(2)}(\mathrm{G})$ be the unique solution of the equation ${\Phi_\mathrm{T}}^{(1)}(\mathrm{G}, \mathrm{T}) = 0$. The derivative $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}$ is the appropriate quantity to investigate the role of genetic regulation in energy homeostasis. It 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. We use formal studies of signs to compare the values of $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}$ at quasistationarity (steady state of genetically non-regulated model) and at stationarity (steady state of the full model). In order to formulate the next result let us denote by B_{eq}, B_{qs} the values at stationarity and at quasi-stationarity of the combination of control coefficients B defined in (4.1).

Proposition 4.1. *Assume that the strong lipolytic condition (4.2) and the stoichiometry condition (4.4) are satisfied. Assume that* $\left(R_\mathrm{T}^{\mathrm{In2}} - R_\mathrm{T}^{\mathrm{Ox2}}\right)_{eq,qs} > 0$ *and* $B_{eq} > B_{qs}$. *Then* $\left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{qs} > \left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq} > 0$.

The proof of this property is a sign study performed with Mathematica (see Additional material in the Appendix). Notice that this proposition is strongly related to the steady state condition introduced in Theorem 4.1 since both assume the strong lipolytic and stoichiometry conditions. As discussed in the previous section, both hypothesis are biologically reasonable.

Comment on the conditions.

- By using derivative computations, we obtain that $\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}$ have the same sign as $R_{\mathrm{T}}^{\mathrm{Ox2}} - R_{\mathrm{T}}^{\mathrm{In2}}$. Then, the condition $\left(R_{\mathrm{T}}^{\mathrm{In2}} - R_{\mathrm{T}}^{\mathrm{Ox2}}\right)_{eq,qs} > 0$ means that PUFAs increase during fasting and decrease during feeding, which is confirmed by experiments of Lee et al. [La04] on wild-type and PPAR-/- mutant murine liver. This suggests that this hypothesis is biologically reasonable.
- Additionally, the condition $B_{eq} > B_{qs}$ is equivalent to
$$B_1 \left(R_{\mathrm{F}_2}^{\mathrm{Ox1}}\right)_{eq} + B_2 \left(R_{\mathrm{F}_2}^{\mathrm{Ko}}\right)_{eq} + B_3 \left(R_{\mathrm{F}_2}^{\mathrm{Sy}}\right)_{eq} + B_4 \frac{\partial \mathrm{Ox2}}{\partial E3} \frac{\partial E3}{\partial \mathrm{F}_2} > 0,$$
with $B_1 > 0$, $B_4 \frac{\partial \mathrm{Ox2}}{\partial E3} \frac{\partial E3}{\partial \mathrm{F}_2} > 0$. 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. We consequently deduce the following:

Biological prediction. Genetic regulation reinforces the energy buffering effect: variations of ATP for a fixed variation of nutriments are less important when genetic regulations exist.

(b) Effect of genetic perturbation Let us consider now the effect of PPAR knock-out on the model. 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. 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 steady state in wild type and mutants of the coefficient B defined in (4.1).

Proposition 4.2. *Assume that the strong lipolytic condition (4.2) and the stoichiometry condition (4.4) are satisfied. Assume also that* $\left(R_{\mathrm{T}}^{\mathrm{In2}} - R_{\mathrm{T}}^{\mathrm{Ox2}}\right)_{eq,qs} > 0$ *and* $B_{WT,eq} > B_{PPAR-/-,eq}$ *then*
$$\left(\tfrac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq,PPAR-/-} > \left(\tfrac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq,WT}, \quad \textit{and} \quad \left|\tfrac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq,PPAR-/-} > \left|\tfrac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq,WT}.$$

As before, this can be checked by symbolic manipulations (see Appendix). Biologically, the condition $B_{WT,eq} > B_{PPAR-/-,eq}$ is equivalent to $B_1 \left(R_{\mathrm{F}_2}^{\mathrm{Ox1}}\right)_{WT,eq} + B_2 \left(R_{\mathrm{F}_2}^{\mathrm{Ko}}\right)_{WT,eq} + B_4 \frac{\partial \mathrm{Ox2}}{\partial E3} \left(\frac{\partial E3}{\partial \mathrm{F}_2}\right)_{WT,eq} > 0$, with $B_1 > 0$, $B_4 \frac{\partial \mathrm{Ox2}}{\partial E3} \left(\frac{\partial E3}{\partial \mathrm{F}_2}\right)_{WT,eq} > 0$. This means that even if B_2 is negative the oxidation genetic control term is large enough to compensate. It follows:

Biological prediction [PPAR -/- mutants] (a) PPAR knock-out reduces energy buffering. (b) The increase of PUFA concentration under fasting is stronger in PPAR knocked-out cells compared to the same increase in wild type cells.

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$^+$04,BGG$^+$09]. 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.

(c) Dynamical predictions. This model also allows to deduce results on the behavior of several metabolites. For instance, we have $\frac{dT^{(2)}}{dG} > 0$, meaning that ATP decreases at fasting (which is not a surprise). Moreover, $\left|\frac{dF_2{}^{(2)}}{dG}\right|_{qs} > \left|\frac{dF_2{}^{(2)}}{dG}\right|_{eq}$, meaning that the curves representing PUFA concentration during fasting must show an overshoot if the input of glucose is a discontinuous step-like decrease: in this case, the model predicts that the increase in PUFA concentration is greater immediately at quasi-stationarity than later at stationarity.

5 Discussion

We have proposed a methodology to build small complexity abstractions that integrate various qualitative aspects of regulated metabolism. As main feature, such abstractions are integrative (main processes together with their various regulation), low complexity abstraction, not dependant on specific numerical values, and allow 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. The catabolic part of this model has been, after exploding some lumped details, successfully used for quantitative predictions on the behavior of fatty acids pools and of the genetic regulation in murine models [BGG$^+$09].

In this paper we have detailed how, using only sign constraints on partial derivatives of elementary fluxes, it is possible to check the possibility of observed properties of the system and to predict others.

Additionally, we have illustrated how this approach allows to reduce complex models into simpler models that have exactly the same steady states in terms of the remaining variables. In the process of reduction we compute symbolically the control coefficients of the reduced model from the derivatives of the elementary fluxes in the full model. The resulting expressions can be used for direct biological predictions as we did in this case study. Finally, full sequences of box equilibrations can provide conditions for uniqueness of the steady state.

Although the full procedure is not yet automatic, it could be done so in the future. An important algorithmic aim is to develop effective algorithms to test the Gale-Nikaido condition from determinant signs or from topological derived conditions. As suggested in Section 2.4, an important step in such algorithms would be to identify and eliminate from the model the non-essential loops which have vanishing contribution to multi-stationarity. Such a method, combining topological criteria and model reduction will be presented elsewhere.

Another problem to solve is the increasing complexity of the expressions of the control coefficients resulting from the reduction. A solution to keep this complexity within fixed bounds has been proposed in [RGZL08] in connection with numerical solutions of quasi-stationarity equations, but similar methods could be applied to symbolic calculations. The idea is to take into account the orders of magnitude of various quantities (say, control coefficients) and to use consistent asymptotic calculations allowing to identify the dominant terms in the solutions of partial steady state equations.

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6 Appendix: additional detailed proofs

6.1 Proof of Theorem 2.1

Proof of Lemma 2.1 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 2.1. From Lemma 2.1, 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: $\mathit{\Delta} = \{\mathbf{X} \in \mathbb{R}^n_+ , \|\mathbf{X}\| \leq R\}$. This domain is a topological ball; let us denote $\mathit{\Sigma}$ its boundary. If $\mathbf{X} \in \mathit{\Sigma}$ and none of its components is 0, then for R large enough, $\mathbf{\Phi}(\mathbf{X})$ points inward $\mathit{\Delta}$, because G is bounded and $\mathit{\Lambda}_i(\mathbf{X})$ tend to infinity with $\mathbf{X}$, hence $\mathit{\Phi}_i(\mathbf{X}) < 0$, 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 $\mathit{\Delta}$. Since the set of points where the property of pointing inwards is open, we can find a smooth ball D contained in $\mathit{\Delta}$ and sufficiently close to it, such that on the boundary of D, the $\mathbf{\Phi}$ points inward D. ■

6.2 Computational details for Theorem 4.1

The derivatives $\frac{\partial \mathrm{A}^{(1)}}{\partial \mathrm{T}}$, $\frac{\partial {\mathrm{F}_1}^{(1)}}{\partial \mathrm{T}}$, $\frac{\partial {\mathrm{F}_2}^{(1)}}{\partial \mathrm{T}}$ are obtained as

$$\frac{\partial}{\partial \mathrm{T}} \begin{pmatrix} \mathrm{A}^{(1)} \\ {\mathrm{F}_1}^{(1)} \\ {\mathrm{F}_2}^{(1)} \end{pmatrix} = -\left(J^{(1)}\right)^{-1} \frac{\partial}{\partial \mathrm{T}} \begin{pmatrix} \mathit{\Phi}_{\mathrm{A}} \\ \mathit{\Phi}_{\mathrm{F}_1} \\ \mathit{\Phi}_{\mathrm{F}_2} \end{pmatrix}.$$

They thus can be expressed by means of fluxes and of control coefficients in the following way:

$$
\begin{aligned}
-\frac{\det(J^{(1)})}{\chi^{tot}_{\mathrm{F}_1}}\frac{\partial \mathrm{A}^{(1)}}{\partial \mathrm{T}} &= \chi^{tot}_{\mathrm{F}_2}\{R^{\mathrm{Gly}}_{\mathrm{T}} - R^{\mathrm{Krb}}_{\mathrm{T}} + n_2[\rho^{\mathrm{Ox2}}_{\mathrm{F}_2}R^{\mathrm{In2}}_{\mathrm{T}} + (1-\rho^{\mathrm{Ox2}}_{\mathrm{F}_2})R^{\mathrm{Ox2}}_{\mathrm{T}}] + \\
&\quad + n_1[(1-\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})(R^{\mathrm{Ox1}}_{\mathrm{T}} + R^{\mathrm{Sy}}_{\mathrm{T}})\rho^{\mathrm{Ox1}}_{\mathrm{F}_1}R^{\mathrm{In1}}_{\mathrm{T}}]\} + (R^{\mathrm{In2}}_{\mathrm{T}} - R^{\mathrm{Ox2}}_{\mathrm{T}})[-R^{\mathrm{Ko}}_{\mathrm{F}_2} \\
&\quad + n_1(R^{\mathrm{Ox1}}_{\mathrm{F}_2} + R^{\mathrm{Sy}}_{\mathrm{F}_2})(1-\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})] \\
-\frac{\det(J^{(1)})}{\chi^{tot}_{\mathrm{A}}}\frac{\partial \mathrm{F}_1{}^{(1)}}{\partial \mathrm{T}} &= \chi^{tot}_{\mathrm{F}_2}\{\rho^{\mathrm{Sy}}_{\mathrm{A}}(R^{\mathrm{Gly}}_{\mathrm{T}} - R^{\mathrm{Krb}}_{\mathrm{T}} + n_2R^{\mathrm{Ox2}}_{\mathrm{T}}) + n_1[R^{\mathrm{In1}}_{\mathrm{T}} - (1-\rho^{\mathrm{Sy}}_{\mathrm{A}})(R^{\mathrm{Ox1}}_{\mathrm{T}} \\
&\quad + R^{\mathrm{Sy}}_{\mathrm{T}})]\} + (R^{\mathrm{Ox2}}_{\mathrm{T}} - R^{\mathrm{In2}}_{\mathrm{T}})[n_1(R^{\mathrm{Ox1}}_{\mathrm{F}_2} + R^{\mathrm{Sy}}_{\mathrm{F}_2}) - n_2R^{\mathrm{Ox2}}_{\mathrm{F}_2} + \rho^{\mathrm{Sy}}_{\mathrm{A}}R^{\mathrm{Ko}}_{\mathrm{F}_2}] \\
\frac{\partial \mathrm{F}_2{}^{(1)}}{\partial \mathrm{T}} &= (\chi^{tot}_{\mathrm{F}_2})^{-1}(R^{\mathrm{Ox2}}_{\mathrm{T}} - R^{\mathrm{In2}}_{\mathrm{T}}) \qquad (6.1)
\end{aligned}
$$

where $-\det(J^{(1)}) = \chi^{tot}_{\mathrm{F}_2}\chi^{tot}_{\mathrm{A}}\chi^{tot}_{\mathrm{F}_1}(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})$. We deduce $\frac{\partial \Phi_{\mathrm{T}}{}^{(1)}}{\partial \mathrm{T}} = [A(R^{\mathrm{Ox1}}_{\mathrm{T}} - R^{\mathrm{In1}}_{\mathrm{T}}) + B(R^{\mathrm{Ox2}}_{\mathrm{T}} - R^{\mathrm{In2}}_{\mathrm{T}}) + C - D]/[n_1(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})]$ where A, B, C, D are combinations of control parameters defined in Eq. (4.1).

We also prove that if the stoichiometry condition (4.4) is fulfilled, then $X > 0, A > 0, B_1 > 0, B_4 > 0, C > 0, D > 0$ with a lengthy but straightforward formal manipulation of (6.1). We have gathered control coefficients into as large as possible positive combinations. As an illustration of how the stoichiometry condition was used let us consider the sign of D. From $\alpha_{\mathrm{O1}} > \alpha_S$, $X/n_1 - \alpha_{\mathrm{O1}}(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}) = (\alpha_{\mathrm{O1}} - \alpha_S)\rho^{\mathrm{Sy}}_{\mathrm{A}} + n_1\alpha_K\rho^{Krebs}_{A} > 0$, $\rho^{\mathrm{Sy}}_{\mathrm{A}} < 1$ and $\rho^{Ox1}_{F_1} < 1$ it follows that $D > 0$.

6.3 Proof of Propositions 4.1 and 4.2

Lemma 6.1. *Let* $\mathrm{F}^{(2)}_2(\mathrm{G}) = \mathrm{F}^{(1)}_2(\mathrm{G}, \mathrm{T}^{(2)}(\mathrm{G}))$. *If the strong lipolytic condition is satisfied, then the sign of* $\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}$ *is equal to the sign of* $R^{\mathrm{Ox2}}_{\mathrm{T}} - R^{\mathrm{In2}}_{\mathrm{T}}$.

Proof. The chain rule gives

$$\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}} = \frac{\partial \mathrm{F}_2{}^{(1)}}{\partial \mathrm{G}} + \frac{\partial \mathrm{F}_2{}^{(1)}}{\partial \mathrm{T}}\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}.$$

Since $\frac{\partial}{\partial \mathrm{G}}\begin{pmatrix}\mathrm{A}^{(1)}\\ \mathrm{F}_1{}^{(1)}\\ \mathrm{F}_2{}^{(1)}\end{pmatrix} = -(J^{(2)})^{-1}\begin{pmatrix}R^{Gly}_{G}\\ 0\\ 0\end{pmatrix} = \frac{R^{Gly}_{G}}{\chi^{tot}_{\mathrm{A}}\chi^{tot}_{\mathrm{F}_1}(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})}\begin{pmatrix}\chi^{tot}_{\mathrm{F}_1}\\ \chi^{\mathrm{Sy}}_{\mathrm{A}}\\ 0\end{pmatrix}$ we have $\frac{\partial \mathrm{F}_2{}^{(1)}}{\partial \mathrm{G}} = 0$. It follows from Eq. (6.1) – computations related to the proof of Theorem 4.1 – that the sign of $\frac{\partial \mathrm{F}_2{}^{(1)}}{\partial \mathrm{T}}$ is the same as the sign of $R^{\mathrm{Ox2}}_{\mathrm{T}} - R^{\mathrm{In2}}_{\mathrm{T}}$. Moreover, if the strong lipolytic condition and the stoichiometry condition are satisfied, then $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}} > 0$. ∎

Proof of Proposition 4.1. The differences between stationarity and quasi-stationarity occur at two levels:

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

$$\left(R^{\mathrm{Sy}}_{\mathrm{F}_2}\right)_{qs} = \left(R^{\mathrm{Ox1}}_{\mathrm{F}_2}\right)_{qs} = \left(R^{\mathrm{Ko}}_{\mathrm{F}_2}\right)_{qs} = 0. \tag{6.2}$$

2. At quasi-stationarity the control of F_2 on its oxidation is only a metabolic substrate effect. Genetic control is added at stationarity. We have $R^{\mathrm{Ox2}}_{\mathrm{F}_2} = \frac{\partial \mathrm{Ox2}}{\partial \mathrm{F}_2} + \frac{\partial \mathrm{E}_3}{\partial \mathrm{F}_2}\frac{\partial \mathrm{Ox2}}{\partial \mathrm{E}_3}$ with $\frac{\partial \mathrm{Ox2}}{\partial \mathrm{E}_3} > 0$, and $\chi^{tot}_{\mathrm{F}_2} = R^{\mathrm{Ox2}}_{\mathrm{F}_2} - \frac{\partial \mathrm{In2}}{\partial \mathrm{F}_2}$, $\rho^{\mathrm{Ox2}}_{\mathrm{F}_2} = (1 - \frac{\frac{\partial \mathrm{In2}}{\partial \mathrm{F}_2}}{R^{\mathrm{Ox2}}_{\mathrm{F}_2}})^{-1}$, with $\frac{\partial \mathrm{In2}}{\partial \mathrm{F}_2} < 0$. Furthermore, $\left(\frac{\partial \mathrm{E}_3}{\partial \mathrm{F}_2}\right)_{eq} > 0$ and $\left(\frac{\partial \mathrm{E}_3}{\partial \mathrm{F}_2}\right)_{qs} = 0$. Hence:

$$\left(R^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{eq} > \left(R^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{qs}, \quad \left(\chi^{tot}_{\mathrm{F}_2}\right)_{eq} > \left(\chi^{tot}_{\mathrm{F}_2}\right)_{qs}, \quad \left(\rho^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{eq} > \left(\rho^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{qs}. \tag{6.3}$$

We easily compute the following

$$\frac{\partial \Phi_{\mathrm{T}}{}^{(1)}}{\partial \mathrm{G}} = \frac{R^{Gly}_G\{n_1\alpha_{\mathrm{K}}\chi^{\mathrm{Krb}}_{\mathrm{A}} + \chi^{tot}_{\mathrm{A}}[n_1\alpha_{\mathrm{G}}(1-\rho^{\mathrm{Ox1}}_{\mathrm{F}_1}\rho^{\mathrm{Sy}}_{\mathrm{A}}) + \alpha_{\mathrm{O}1}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1}\rho^{\mathrm{Sy}}_{\mathrm{A}} - \alpha_S\rho^{\mathrm{Sy}}_{\mathrm{A}}]\}}{n_1\chi^{tot}_{\mathrm{A}}(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})}$$

We deduce that $\frac{\partial \Phi_{\mathrm{T}}{}^{(1)}}{\partial \mathrm{G}}$ is the same at stationarity and at quasi-stationarity. From Theorem 4.1, it follows :

$$\frac{\partial \Phi_{\mathrm{T}}{}^{(1)}}{\partial \mathrm{T}} = \mathcal{R} - \frac{B}{n_1(1-\rho^{\mathrm{Sy}}_{\mathrm{A}}\rho^{\mathrm{Ox1}}_{\mathrm{F}_1})}(R^{\mathrm{In2}}_{\mathrm{T}} - R^{\mathrm{Ox2}}_{\mathrm{T}}), \tag{6.4}$$

where $\mathcal{R}$ is a term not changing from quasi-stationarity to stationarity and the expression of B is given in (4.1).

It remains to notice that $\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}} = -\left(\frac{\partial \Phi_{\mathrm{T}}{}^{(1)}}{\partial \mathrm{G}}\right) / \left(\frac{\partial \Phi_{\mathrm{T}}{}^{(2)}}{\partial \mathrm{T}}\right)$ and $B_{qs} < B_{eq}$ to conclude $\left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{qs} > \left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{eq}$. From $\frac{d\mathrm{F}_2^{(2)}}{d\mathrm{G}} = -\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}(R^{\mathrm{In2}}_{\mathrm{T}} - R^{\mathrm{Ox2}}_{\mathrm{T}})/\chi^{tot}_{\mathrm{F}_2}$ it also follows that $\left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{qs} > \left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq}$. ∎

Proof of Proposition 4.2 We follows closely the proof of Prop. 4.1. The differences between $PPAR-/-$ and WT cells occur at two levels:

$$\left(R^{\mathrm{Ox1}}_{\mathrm{F}_2}\right)_{PPAR-/-} = \left(R^{\mathrm{Ko}}_{\mathrm{F}_2}\right)_{PPAR-/-} = 0 \tag{6.5}$$

$$\left(R^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{WT,eq} > \left(R^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{PPAR-/-,eq}, \left(\chi^{tot}_{\mathrm{F}_2}\right)_{WT,eq} > \left(\chi^{tot}_{\mathrm{F}_2}\right)_{PPAR-/-,eq},$$

$$\left(\rho^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{WT,eq} > \left(\rho^{\mathrm{Ox2}}_{\mathrm{F}_2}\right)_{PPAR-/-,eq} \tag{6.6}$$

If $B_{WT,eq} > B_{PPAR-/-,eq}$, it follows (along the same lines as the proof of Prop. 4.1) that
$\left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{PPAR-/-,eq} > \left(\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}\right)_{WT,eq}$. From $\frac{d\mathrm{F}_2^{(2)}}{d\mathrm{G}} = -\frac{d\mathrm{T}^{(2)}}{d\mathrm{G}}(R^{\mathrm{In2}}_{\mathrm{T}} - R^{\mathrm{Ox2}}_{\mathrm{T}})/\chi^{tot}_{\mathrm{F}_2}$ and Eq.(6.6) it follows that $\left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq,PPAR-/-} > \left|\frac{d\mathrm{F}_2{}^{(2)}}{d\mathrm{G}}\right|_{eq,WT}$. ∎