Equilibria and their changes for genetically regulated lipid metabolism in liver

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Abstract. *We introduce a general approach that can be used for the study of equilibria and of changes of equilibria (either discontinuous that we call switches or continuous that we call shifts) of mixed (genetic and metabolic) networks. We define equilibria as stable steady states. Using this approach for the hepatic fatty acids metabolism, we show that the increase of unsaturated fatty acids under fasting is an indirect proof that changes in genetically controlled lipid metabolism are shifts.*

Introduction

Equilibrium shift and equilibrium switch Metabolic systems have a rather high level of complexity due to the interplay between homeostasis and a certain flexibility allowing for several functioning modes. An example is given by the lipid metabolism in hepatocytes, for which two functioning antagonist modes exist: the lipogenesis that produces fatty acids and the oxidation that burns fatty acids and produce energy. The choice of the functioning mode depends on external conditions: a lack of food stimulates $\beta-$oxidation, while normal feed induces lipogenesis. Another example is the lactose operon in the bacterium E.Coli. The two functioning modes correspond to the production and the non-production of the enzymes necessary for the decomposition of lactose into simpler molecules.

A way to sustain this dialectics is *multistationarity*. A multistationary system is able to commute between (at least two) stable stationary functioning modes (equilibria) under a change of the external conditions by *equilibrium switch*, such as the functioning of the lactose operon in E.Coli. For this bacterium the sharp separation between consumption and non-consumption has a functional justification : it saves resources; the necessary enzymes are produced only when lactose is present [6]. By analogy, we may think that under fasting, liver cells switch from a lipogenesis mode to an oxidation mode.

The alternative to multistationarity is the uniqueness of the equilibrium: even without multistationarity, metabolic systems could change functioning modes under changes of external conditions by *equilibrium shift*. The main difference between *equilibrium switch* and *equilibrium shift* is that the first is discontinuous (equilibrium changes suddenly at switching values of external parameters) and hysteretic (under reversed changes of external conditions the equilibrium follows distinct paths; switching occurs at different values of increasing and decreasing external parameters) while the latter is continuous and reversible [5]. Equilibrium switch is efficient in saving resources, because these are produced only on demand. As a counterpart it is less flexible because it needs a minimal threshold stimulus to act and because tuning is not possible (the response is of the binary type). For the equilibrium shift there is no threshold and tuning of resources is possible (the response is graded). A mathematical discussion of the differences between shifts and switches is given in the Appendix**??**.

Positive regulation loops and number of equilibria The multistationarity of the lactose operon is related to a positive loop in the gene regulation network associated with Lactose. The inducer I, which is a molecule related to the Lactose enters the cell and cancels the action of the inhibitor of the transcription promoter P. Active transcription leads to production of the enzyme $\beta - galactosidase$ E that breaks lactose into simpler molecules and also of permease P that allows more inducer to enter the cell. Thus, I has a positive action on P and P has a positive action on I, that is, the operon lactose has a positive regulation loop. As a consequence the operon can work in two states : ON (high I, high E,P), and OFF (low I, no E,P). The passage from one state to the other is an equilibrium switch and is produced by changing the exterior concentration of lactose.

In general, a conjecture of R. Thomas [1,3,4] proved by C. Soulé [2] states that a necessary condition for the multistationarity of gene networks is the existence of at least a positive regulation loop; however the positive loop is not a sufficient condition for multistationarity. The proof of Soulé still holds concerning metabolic changes: if there is no positive regulation loop, one could only have equilibrium shift; conversely, the existence of a positive regulation loop does not automatically mean that one has equilibrium switch. Further analysis is necessary in this case. In this paper we provide an example of a genetically regulated metabolic network that has a positive regulation loop and no multistationarity.

Lipid metabolism The lipid metabolism in superior organisms have complex regulations, among which hormonal and nervous regulation signals, intrinsic regulations related to metabolic biochemistry and a regulation of fatty acids on genes controlling their metabolism. Consequently, like the lactose metabolism in E.Coli, lipid metabolism is controlled by a mixed regulation network whose nodes are metabolites as well as genetic variables. The first question we wish to answer is whether the whole of these regulations produce multistationarity in lipid metabolism.

A quick inspection of the regulations shows that lipid metabolism regulation network contains positive loops. Some of the fatty acids can be synthesized de novo from acetyl-CoA. There is a positive path from acetyl-coA to fatty acids. Conversely, the oxidative pathway produces acetyl-coA from fatty acids, closing the positive loop. This positive loop has metabolic origin and exists independently of the genetic regulation. Hence, in this situation Thomas criterion (generalized to mixed networks) can not reject multistationarity. By a different approach, we shall be able to find sufficient conditions for the uniqueness of equilibrium, implying equilibrium shift. Our main result shall be summarized as follows.

> *The mixed regulation network of lipid metabolism in liver has a positive regulation loop. A sufficient condition for unique equilibrium (hence, absence of multistationnarity) is that fasting increases the concentration of fatty acids (polyunsaturated, hence obtained from diet or all other synthesized de novo) in liver. This hypothesis is plausible because it coincides to the experimental observation of fatty acids concentration increase in rat liver after 72h of fasting [12].*

Among the fatty acids only a special class (certain polyunsaturated fatty acids, PUFA) interfere with genes [11]. PUFA controls de novo lipogenesis or synthesis and OXIdation of themselves and of other fatty acids by interacting with nuclear receptors regulating transcription of genes coding for enzymes involved in the corresponding pathways. Interestingly, PUFA can not be synthesized de novo and must be produced from essential fatty acids taken from the diet. The second question we wish to answer is about the importance of PUFA in lipid metabolism.

Mathematical method Our approach starts from a differential model of mixed regulation networks and imposes stationarity. The stationarity equations are solved in several steps. At each step we consider a group of equations that we call box or module. We call this procedure *implicit differential static model* (IDSM), because at each step some of the variables are expressed as functions of the others via the implicit function theorem. IDSM can conveniently identify the set of equilibria and the changes of equilibria induced by changes of external parameters. In our analysis we shall be able to keep a high level of generality and we shall not impose particular forms of the interaction functions. This also implies that we do not give particular values to any parameters that may be involved in these functions. The conditions that we obtain involve only partial derivatives of these functions. Thus, our approach is

close to the one used in classical equilibrium thermodynamics [7] in order to extract relations between thermodynamic coefficients (derivatives of the state variables) from the equations of state (equations describing equilibrium).

Although it may seem difficult to understand how time could be present in a static approach, time scale differences between genetic and metabolic regulation can be taken into account into IDSM. Genetic regulation becomes effective only when transcriptional machinery is activated and processed. This is very slow compared to rapid metabolic changes. It may actually take tens of minutes or hours to perform the necessary re-adjustments. In IDSM we may take this into account as following : on short time scales genetic variables are constant and not equilibrated. It is only on long time scales that their equilibration equations can be used. This means that genetic regulations are absent on short time scales and present only on long time scales. In lipid metabolism, if under fasting the shift from a lipogenesis dominated state to an oxidation dominated state was under the control of a slow genetic mechanism only, the hungry cell would die. We shall argue that a rapid metabolic and hormonal mechanism exists and that the role of genetic regulation is to reinforce it.

1 Implicit differential static model IDSM

We present here a general approach that can be used for the study of equilibria and of changes of equilibria (either switches or shifts) of mixed (genetic and metabolic) networks. 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. By equilibria we do not mean thermodynamic equilibria in which all fluxes vanish and which correspond to the death of the cell, but we mean stationary states in which fluxes do not vanish (they simply do not change in time). Restricting our analysis to stationary states is a weakness of this approach that can not identify more general attractors such as limit cycles or chaotic attractors. Our opinion is that this restriction is not very important since stationary states are normal for controlled metabolism while limit cycles and chaotic attractors are rather exceptional.

1.1 Mixed (genetically controlled metabolisms) differential system

Mixed differential system A differential model of a mixed system, that is, a is genetically controlled metabolism, is given by three vectors $(\mathbf{X}, \mathbf{Y}, \mathbf{p})$ and two functions $\mathbf{\Phi}$ and $\mathbf{F}$. The vector $\mathbf{X}$ denotes the metabolic variables, the vector $\mathbf{Y}$ denotes the genetic variables, and $\mathbf{p}$ stands for a set of external parameters. The function $\mathbf{\Phi}$ describes the variations of metabolic variables and $\mathbf{F}$ describes the variations of genetic variables. Consequently, the dynamics of these variables is supposed to follow a system of differential equations:

$$\frac{d\mathbf{X}}{dt} = \mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p})$$
$$\frac{d\mathbf{Y}}{dt} = \mathbf{F}(\mathbf{X}, \mathbf{Y}, \mathbf{p})$$

Difference between derivative functions There are physical differences between $\mathbf{\Phi}$, the time derivatives of metabolic variables and $\mathbf{F}$, the time derivatives of genetic variables.

- The first ones are fluxes and may obey to conservation laws, while the latter are generally non-conservative. Nonetheless this difference is relevant only for the construction of the model (in particular for identifying the relations among fluxes) and can be forgotten in the study of equilibria once that the model has been built.
- A more important difference involves time scales. Many genetic variables vary on time scales generally much longer than any of the metabolic variables. This is important not only for dynamics but also for the study of equilibria or to be more precise of what experiments may consider as equilibria.

A typical experiment consists in changing one or several external parameters and observe the state after a waiting time τ that is supposed to be long enough to ensure the reach of equilibrium.

Quasi-stationary state If τ is shorter than genetic time scales but longer than metabolic time scales, than one observes a state in which only metabolic variables are equilibrated. Hence, a *quasi-stationary state for a mixed differential model* is defined by:

$$\mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0 \tag{1.1}$$

$$\mathbf{Y} = \text{const.} \tag{1.2}$$

Equilibrium state If τ is longer than both metabolic and genetic time scales, complete equilibrium is obtained. Hence, a *complete equilibrium state for a mixed differential model* is defined by the equations :

$$\mathbf{\Phi}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0 \tag{1.3}$$

$$\mathbf{F}(\mathbf{X}, \mathbf{Y}, \mathbf{p}) = 0 \tag{1.4}$$

1.2 Implicit differential static model

At complete equilibrium Eq. (1.4) can be used to eliminate genetic variables and defines them as implicit functions of the metabolites and of the external parameters: $\mathbf{Y} = \mathbf{G}(\mathbf{X}, \mathbf{p})$. The elimination of genetic variables is allowed locally by the implicit function theorem supposing that the matrix of partial derivatives $\frac{\partial F_i}{\partial Y_j}$ is invertible which is true in points that are non-critical in genetic variables. Critical points correspond to bifurcations and multistationarity produced by genetic regulation alone and need a special treatment. In the model that we study in this paper genetic regulation alone can not produce bifurcations; these can arrive from the coupling between genetic regulation and metabolism.

The resulting implicit functions can be used to express the fluxes as functions of the metabolites and of the external parameters only and to reduce the equilibrium equations to :

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

At quasi-stationarity Eq. (1.4) must be replaced by Eq. (1.2) and the equilibrium equations are Eq. (1.1) where genetic variables are constants (meaning that they do not change when external parameters change).

We say that an equilibrium state exists if Eq. (1.5) has a solution in $\mathbf{X}$. We say that a quasi-stationary state exists if Eq. (1.1) has a solution.

Thus, both complete equilibrium and quasi-stationarity lead to a reduced model that we call implicit differential static model (IDSM).

Definition 1 *Let* $(\mathbf{X}, \mathbf{Y}, \mathbf{p}, \mathbf{\Phi}, \mathbf{F})$ *be a mixed differential system with no critical genetic variables, that is, the matrix* $(\frac{\partial F_i}{\partial Y_j})_{i,j}$ *is always invertible.*

An equilibrium implicit differential static model (IDSM) *(respectively quasi-stationary IDSM) is a triplet* $(\mathbf{X}, \mathbf{p}, \overline{\mathbf{\Phi}})$ *where* $\overline{\mathbf{\Phi}}$ *denotes a function such that* $\overline{\mathbf{\Phi}}(\mathbf{X}, \mathbf{p}) = 0$ *is a system of equations for the metabolites levels* $\mathbf{X}$ *at equilibrium state (respectively quasi-stationary state) expressing their fluxes as functions of their levels after eliminating genetic variables, and canceling these fluxes in order to find equilibria.*

In IDSM, the equilibrium equations consist in canceling fluxes that are functions of metabolites and external parameters only. At complete equilibrium the dependence of the fluxes on the metabolites takes into account genetic regulations. At quasi-stationarity this dependence is purely metabolic.

One could notice that the classical singular perturbations approach to two scales dynamics [8] performs the reduction in the opposite direction. In order to study the dynamics of slow genetic variables, one should eliminate the metabolic variables and express the time derivatives of the genetic variables as functions of the genetic variables and of the external parameters. Here we are not interested in dynamics, but in equilibria. Our approach is static and the order of eliminations of variables does not matter because the order of successive eliminations of variables does not matter when one solves any system of equations. Furthermore, our definition of quasi-stationarity (Eqs. (1.1,1.2)) corresponds only to the start and not to the entire long time dynamics as it is customary in singular perturbation dynamics [8].

1.3 Box reduction of IDSM

IDSM is a system of equations for the metabolites levels $\mathbf{X}$ at equilibrium. In order to solve this system we can continue our elimination of variables. The purpose is to express the metabolites levels as functions of the external parameters $\mathbf{X} = \mathbf{M}(\mathbf{p})$. As already noticed the order of eliminations is arbitrary. Therefore we can choose to define groups of variables (boxes) that will be eliminated together. The choice of the boxes can be arbitrary, but we may group together metabolites that fulfill a well-defined biological function or which have simple mutual relations such as antagonism.

Box of an IDSM We call *box of an IDSM* $(\mathbf{\Phi}, \mathbf{X})$ a subset $\mathbf{X^{(i)}}$ of the set of metabolites $\mathbf{X}$. The variables in $\mathbf{X^{(i)}}$ are called *internal variables*. The metabolites $\mathbf{X^{(e)}}$ that are not internal to the box are called *external variables*.

We call *box equilibration* the elimination of internal variables by setting the fluxes of these variables to zero, that is, the equation $\mathbf{\Phi^{(i)}}(\mathbf{X^{(i)}}, \mathbf{X^{(e)}}, \mathbf{p}) = 0$, with variables $\mathbf{X^{(i)}}$, where $\mathbf{\Phi^{(i)}}$ denotes the components of $\mathbf{\Phi}$ that correspond to $\mathbf{X^{(i)}}$.

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 iteration of the following operations:

1. Define $\mathbf{X}_o = \mathbf{X}$, and $\mathbf{\Phi_o}(\mathbf{X}_o) = \mathbf{\Phi}(\mathbf{X})$.
2. At n-th iteration, $\mathbf{\Phi_n^{(i)}}(\mathbf{X_n^{(i)}}, \mathbf{X_n^{(e)}}, \mathbf{p}) = 0$, where $\mathbf{X_n^{(i)}}$ and $\mathbf{X_n^{(e)}}$ are the internal and the external variables for the box n.
3. Eliminate the internal variables $\mathbf{X_n^{(i)}} = \mathcal{M}_\mathbf{n}(\mathbf{X_n^{(e)}}, \mathbf{p})$ where the function $\mathcal{M}_\mathbf{n}$ is found by solving the system provided by the previous operation. Notice that the solution might not be unique, that is $\mathcal{M}_\mathbf{n}$ is not necessarily univoque.
4. Define $\mathbf{X_{n+1}} = \mathbf{X_n^{(e)}}$, and $\mathbf{\Phi_{n+1}} = \mathbf{\Phi_n^{(e)}}(\mathcal{M}_\mathbf{n}(\mathbf{X_n^{(e)}}, \mathbf{p}), \mathbf{X_n^{(e)}}, \mathbf{p})$.

A sequence of N box equilibrations is said to be *complete* if all variables are equilibrated i.e. if

$$\mathbf{X} = \mathbf{X_1^{(i)}} \oplus \mathbf{X_2^{(i)}} \oplus \ldots \oplus \mathbf{X_N^{(i)}}.$$

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 $\{\mathcal{M}_\mathbf{n}\}_{n=1,N}$.

Condition for equilibrium states The existence and properties of equilibrium states relatively to box equilibrations are straightforward.

Property 1

- *An equilibrium or a quasi-stationary state exists if each one of the equations* $\mathbf{\Phi_n^{(i)}}(\mathbf{X_n^{(i)}}, \mathbf{X_n^{(e)}}, \mathbf{p}) = 0$ *have a solution for a complete sequence of box equilibrations.*
- *The function* $\mathbf{M}$ *is univoque (to one* $\mathbf{p}$ *corresponds a single value of* $\mathbf{M}$*) and therefore the equilibrium is unique if each one of the function* $\mathbf{M_n}$ *is univoque for a complete sequence of box equilibrations.*
- *There is a equilibrium or a quasi-stationary state, only if the equations* $\mathbf{\Phi_n^{(i)}}(\mathbf{X_n^{(i)}}, \mathbf{X_n^{(e)}}, \mathbf{p}) = 0$ *have solutions for any complete sequence of box equilibrations.*
- *The function* $\mathbf{M}$ *is univoque only if each one of the functions* $\mathbf{M_n}$ *is univoque for any complete sequence of box equilibrations.*

The "if" part of the properties are useful to prove the uniqueness of equilibrium: it is enough to choose a complete sequence of box equilibrations and show that at each step the equations $\mathbf{\Phi_n^{(i)}}(\mathbf{X_n^{(i)}}, \mathbf{X_n^{(e)}}, \mathbf{p}) = 0$ have a unique solution for $\mathbf{X_n^{(i)}}$. The "only if" part of the properties are useful to prove the presence of multistationarity: it is enough to find a box such that the equations $\mathbf{\Phi_n^{(i)}}(\mathbf{X_n^{(i)}}, \mathbf{X_n^{(e)}}, \mathbf{p}) = 0$ have multiple solutions for $\mathbf{X_n^{(i)}}$.

1.4 Variation of metabolites

Let us consider that we have a solution $\mathbf{X} = \mathcal{M}(\mathbf{p})$ for the equilibrium equations.

Equilibrium shift means that the function $\mathcal{M}$ is univoque and smooth and a variation of $\mathbf{X}$ under a variation of $\mathbf{p}$ could be found by integration :

$$\Delta\mathbf{X} = \int_{\mathbf{p}}^{\mathbf{p}+\Delta\mathbf{p}} \frac{d\mathcal{M}}{d\mathbf{p}} d\mathbf{p} \tag{1.6}$$

Equilibrium switch means that the function $\mathcal{M}$ has several branches. Rather general arguments (see Appendix) show in this case that the equilibrium shift follows a smooth branch up to a bifurcation (usually of the saddle-node type) and then jumps discontinuously to another branch. Eq.1.6 remains valid where $\mathcal{M}$ is a piecewise differentiable union of branches.

Hence, in both situations the sign of the partial derivative $\frac{\partial \mathcal{M}_\mathbf{i}}{\partial \mathbf{p_j}}$ will inform on the sign of the variation of $\mathbf{X_i}$ under a positive variation of $\mathbf{p_j}$.

2 A model for genetically regulated lipid metabolism in liver

Our aim is to define a model of the lipid metabolism in liver and its regulations that fit with experiments and knowledge on that subject.

2.1 Non genetically regulated model

Metabolic variables We select most important metabolites implied in the lipid metabolism in liver.

1. M stands for *Acetyl-CoA*, the first brick for building fatty acids in mitochondria. M is consumed in lipogenesis in hepatocytes, produced in oxidation.
2. A_1 denotes all de novo synthesized *fatty acids* within the cell. It is opposed to M (produced in lipogenesis, consumed in oxidation).
3. A_2 are polyunsaturated fatty acids that interact with genetic regulation. For the purposes of this paper we do not need to distinguish more than two classes of fatty acids. Our classification is consistent with the suggestion that (see [11] for a review) de novo synthesized fatty acids have little or no interaction with genetic regulation.
4. T stands for the ratio *ATP/ADP*. It expresses the energy that the cell has at its disposal.
5. P denotes the *pyruvate* which is a connection node between glucose and Acetyl-CoA.
6. G stands for the *glucose*. This models the input parameter (food).

Fluxes Known fluxes relations between these products are summarized in Fig.2.1. These fluxes stand for the main processes of the lipid metabolism in liver. Notice that although we have indicated a direction for each flux, some of them are reversible.

1. Glycolisis GLY produces pyruvate from glucose.
2. The pyruvate transforms into Acetyl-CoA (pyruvate dehydrogenase reaction PDH), that is used either to produce energy for cellular needs (Krebs cycle $KREBS$), or to transfer energy to the outside (ketone bodies exit $KOUT$).
3. An intermediate metabolite of the Krebs cycle (citrate) is the input to the lipogenesis of A_1 (lipogenesis SYN).
4. Fatty acids A_1 exit the liver cell and can be stored as triacylglycerols in adipocytes. Conversely, adipocytes can be broken down and fatty acids are released in the bloodstream (lipolysis) and enter the cells. The entering (exiting) flux corresponds to a positive (negative) value of $AIN1$. Essential precursors of polyunsaturated fatty acids A_2 can also enter the cell from the blood stream following diet or lipolysis. The corresponding reversible flux is called AIN_2. Although not discussed explicitly here, this flux includes also a synthetic pathway consisting of desaturation and elongation of essential fatty acids.
5. All fatty acids can be burned burned in order to produce energy and to recover Acetyl-CoA ($\beta-$oxidation $OXI1$ and $OXI2$).

6. $CONS(T)$ expresses the energy (ATP) the cell has to consume to live.
7. Degradation of metabolites $DEGP$, $DEGM$, $DEGA1$, $DEGA2$ is included in the model. Even if it is negligible on the timescale of the metabolic processes, it can no longer be neglected on the genetic timescale.

Flux	Symbol	Effect	Reversible
Glycolysis	GLY	consumes G, produces P	no
P degradation	$DEGP$	consumes P	no
Pyruvate dehydrogenase reaction	PDH	consumes P, produces M	no
Krebs cycle	$KREBS$	consumes M, produces T	no
Ketone body exit	$KOUT$	consumes M	no
Lipogenesis	SYN	consumes M, produces A_1	no
M degradation	$DEGM = \chi_M M$	consumes M	no
$\beta-$oxidation	$OXI1,2$	consumes $A_{1,2}$, produces M, T	yes
Fatty acids enter	$AIN1,2$	produces $A_{1,2}$	yes
$A_{1,2}$ degradation	$DEGA1,2 = \chi_{A_{1,2}} A_{1,2}$	consumes $A_{1,2}$	no
ATP consumption	$CONS(T)$	consumes T	no

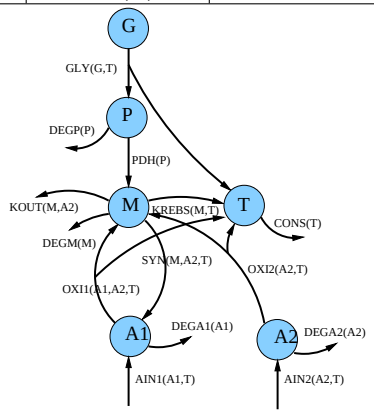


Fig. 2.1 Fluxes relations between main metabolites implied in lipid metabolism

Differential model A differential model is obtained by gathering the fluxes related to each metabolites.

$$\begin{aligned}
\frac{dP}{dt} &= GLY(G,T) - DEGP(P) - PDH(P) \\
\frac{dA_1}{dt} &= SYN(M,A_1,A_2,T) - OXI1(A_1,A_2,T) + AIN1(A_1,T) - DEGA1(A_1) \\
\frac{dA_2}{dt} &= -OXI2(A_2,T) + AIN2(A_2,T) - DEGA2(A_2) \\
\frac{dM}{dt} &= PDH(P) + OXI1(A_1,A_2,T) + OXI2(A_2,T) - KREBS(M,T) \\
&\quad -KOUT(M,A_2) - SYN(M,A_1,A_2,T) - DEGM(M) \\
\frac{dT}{dt} &= \alpha_G GLY(G,T) + \alpha_K KREBS(M,T) + \alpha_{O1} OXI1(M,A_1,A_2,T) + \alpha_{O2} OXI2(M,A_2,T) - CONS(T)
\end{aligned} \tag{2.1}$$

Notice that the relation for the flux of T is different from the others. Indeed, proportionality coefficients have to be introduced such that the flux received by a metabolite can be disconnected from the flux received by T. For instance, if $GLY(G,T)$ goes to P, T receives a flux that is proportional to $GLY(G,T)$ but there is no reason for these fluxes to be equal. Contrarily, the flux $SYN(M,A_1,A_2,T)$ that goes to A_1 is exactly the opposite of the flux related to the lipogenesis process that is lost by M.

Functioning modes There are two functioning antagonist modes of the lipid metabolism: lipogenesis and storage that produce reserves, lipolysis and oxidation that burn reserves and produce energy.

The choice of the functioning mode may depend on the type of cell and the environment conditions: a lack of food (that is a long time decrease of G) should stimulate lipolysis and lead to $\beta-$oxidation; normal feed (G equals to a constant) should induce lipogenesis.

The reason why we restrict our model to liver cells is that our discussion is based on the existence of these two different functioning modes. Hepatocyte (liver) cells have the specificity to ensure lipogenesis, although all cells are capable of $\beta-$oxidation.

2.2 Regulations

Three classes of regulations are considered in this model: hormonal regulation, metabolic biochemistry regulation, action of certain fatty acids on genes that regulate lipid metabolism.

Hormonal regulation Important regulation signals are hormonal. The main hormonal regulation are performed by the balance glucagon/insulin. When this is in favor of insulin, lipolysis and oxidation are suppressed and lipogenesis is induced. When this is in favor of glucagon, lipolysis and oxidation are stimulated and lipogenesis is blocked.

The variable T expresses the energy the cell has at its disposal and play an important role in hormonal regulation. Its changes induce the hormonal response of the organism. This response integrates signals from several cells. A complete model should take into account the fact that in higher organisms it is not a single cell, but an entire population that is responding to a stimulus. Here we consider that all cells act as a single one (synchronism).

We consider the following hormonal regulations :

1. $AIN1, 2$, the fatty acids incoming/outgoing flux depends on A but also on T. It can be positive (A enters) or negative (A exits). A drop in T will inform the organism on the lack of energy and some hormones (glucagon, epinephrine [16]) will trigger lipolysis: fatty acids enter the cell, AIN becomes positive and increases. This means that $\frac{\partial AIN}{\partial T} < 0$. There is a similar effect on oxidation $\frac{\partial OXI1,2}{\partial T} < 0$.
2. Similarly, an increase in T will trigger insulin which induces lipogenesis, blocks lipolysis and oxidation. Therefore $\frac{\partial SYN}{\partial T} > 0$. The signs of these partial derivatives are also consequences of purely metabolic biochemical control, see next.

Metabolic regulations Another type of regulation is intrinsic to metabolic biochemistry. Substrates and products stimulate and inhibit reactions consuming and producing them, respectively. Even far from thermodynamical equilibrium, metabolic reactions seem to follow Le Chatelier principle from equilibrium thermodynamics (effects turn against causes [7], hence regulation loops are negative) that provides stability and allows avoiding multistationarity.

Metabolic regulations that we consider are either substrate or product effects: a substrate (product) of a pathway increases (decreases) the associated flux. The case of T is special, because this variable represents in fact the antagonist couple ATP/ADP. When ADP is the substrate of a reaction, ATP is a product and the flux decreases with T, either by substrate or by product effect.

Substrate effect	$\frac{\partial SYN}{\partial M} > 0$, $\frac{\partial GLY}{\partial G} > 0$, $\frac{\partial OXI1,2}{\partial A_{1,2}} > 0$, $\frac{\partial KOUT}{\partial M} > 0$, $\frac{\partial PDH}{\partial P} > 0$, $\frac{\partial KREBS}{\partial P} > 0$
Substrate/product	$\frac{\partial CONS}{\partial T} > 0$, $\frac{\partial KREBS}{\partial T} < 0$
Substrate/product and hormonal	$\frac{\partial OXI1,2}{\partial T} < 0$, $\frac{\partial GLY}{\partial T} < 0$, $\frac{\partial SYN}{\partial T} > 0$, $\frac{\partial AIN2}{\partial T} < 0$
Hormonal regulation	$\frac{\partial AIN1}{\partial T} < 0$

Table 2.1 Metabolic regulations in lipid metabolism

Genetic regulations Several recent works indicate that polyunsaturated fatty acids (PUFA) can regulate in a positive or negative manner the expression of different genes controlling their metabolism (for reviews see [10,13,11]). A good part of the known regulation mechanisms implies transcription factors such as nuclear receptors. One of the mechanisms that were proposed is that PUFA can bind and regulate the activity of several members of the steroid-thyroid superfamily of nuclear receptors as 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. Another mechanism is that PUFA regulate the nuclear abundance of transcriprion factors such as SREBP-1 via the turnover of its mRNA and also via its proteolytic processing which is specific of SREBP's family. These are transcription factors that bind (most often as dimers) to a specific consensus sequence on the DNA chain. Transcription of the corresponding genes is blocked by complex repressors and/or by histones that wrap nuclear chromatin in a way that makes it inaccessible to transcription. Fatty acids could activate transcription either directly by repelling repressors and recruiting activators ore indirectly by inducing chromatin remodeling. The activation hypothesis proposed in [10] is not the only possibility to explain the action of fatty acids on genes. There is also an inhibition hypothesis considering that fatty acids prevent nuclear receptors from forming heterodimers with RXR (retinoid X receptor), and therefore block their activity as transcription factors.

In the present state of the art ([10,13,11]), the following facts seem quite well established.

1. Specificity. The action of fatty acids on genes is specific of their structure, depending on the length of the chain and on the degree of saturation. Thus, in mammals mainly long chain PUFA interfere with genetic regulation. Chromatin remodeling and the specificity of the biochemical interactions with the nuclear receptors may be responsible for this effect.
2. Down-regulation. PUFA (A_2) inhibit their synthesis from essential fatty acids as well as the lipogenesis of other FA (A_1) via the LXRα-SREBP-1 regulation path. They prevent LXR from forming a heterodimer with RXR . This diminishes the concentration of SREB-1 transcription factors important for lipid lipogenesis. The flux SYN decreases with A_2.
3. Up-regulation. PUFA stimulate their oxidation as well as the oxidation of A_1 since they activate PPAR. The detailed mechanism is not known : it can be either indirect (active PPAR is a heterodimer with RXR; preventing LXR/RXR formation fatty acids shift the equilibrium toward PPAR/RXR formation) or direct stimulation of transcriptional role of PPAR. The fluxes $OXI1,2$ increase with A_2.
4. Ketone exit 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 [12]. Therefore the flux $KOUT$ should increase with A_2.

Genetic variables Since the genetic interactions between metabolites and fluxes is not direct, we need to give an abstraction for the genetic regulation variables.

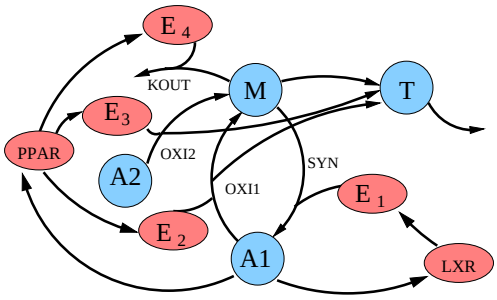
1. $PPAR$, LXR stand for the active forms of the nuclear receptors PPAR and LXR. LXR represents in a very simplified way the regulation path LXRα-SREBP-1.
2. $E1$, $E2$, $E3$ design in a simplified model the set of enzymes whose production is controlled by LXR and $PPAR$ and that are involved in the fluxes SYN,OXI,$KOUT$.

The relations between these variables were detailed above. They are summarized in Fig. 2.2. From these relations is deduced a differential system that describes the dependancies of genetic variables fluxes. This simple system is not adequate for an accurate description of the dynamics, but is sufficient for the study of changes of equilibria, that is our purpose. Notice that the introduction of genetic variables implies that the fluxes SYN,$OXI1,2$,$KOUT$ becomes function of the metabolites and of $E1,E2,E3,E4$.

The following inequalities translate biological knowledge on genetic regulation that was detailed above.

2.3 IDSM for regulated lipid metabolism

The genetic variables are $\mathbf{Y} = (PPAR, LXR, E1, E2, E3, E4)$, the metabolites are $\mathbf{X} = (M, A_1, A_2, T, P)$, and the external parameter is G. The complete regulated differential model consists of Eq.2.1 in which



$$\begin{aligned}
\frac{dPPAR}{dt} &= F_1(A_2, PPAR) \\
\frac{dLXR}{dt} &= F_2(A_2, LXR) \\
\frac{dE1}{dt} &= F_3(LXR, E1) \\
\frac{dE2}{dt} &= F_4(PPAR, E2) \\
\frac{dE3}{dt} &= F_5(PPAR, E3) \\
\frac{dE4}{dt} &= F_6(PPAR, E4)
\end{aligned} \tag{2.2}$$

$$SYN = \Phi_1(M, T, E1) \tag{2.3}$$
$$OXI1 = \Phi_2(A_1, T, E2) \tag{2.4}$$
$$OXI2 = \Phi_3(A_2, T, E3) \tag{2.5}$$
$$KOUT = \Phi_4(M, E4) \tag{2.6}$$

Fig. 2.2 Genetic regulations of lipid metabolism

PUFA activates PPARα and inhibates active LXRα and SREBP-1	$\frac{\partial F_1}{\partial A_2} > 0$, $\frac{\partial F_2}{\partial A_2} < 0$
LXRα and SREBP-1 triggers $E1$ production	$\frac{\partial F_3}{\partial LXR} > 0$
PPARα triggers $E2$,$E3$,$E4$ production	$\frac{\partial F_i}{\partial PPAR} > 0$, $i = 4, 6$
Negative self-regulation due to degradation effects	$\frac{\partial F_1}{\partial PPAR} < 0$, $\frac{\partial F_2}{\partial LXR} < 0$, $\frac{\partial F_3}{\partial E1} < 0$, $\frac{\partial F_4}{\partial E2} < 0$, $\frac{\partial F_5}{\partial E3} < 0$, $\frac{\partial F_6}{\partial E4} < 0$
Ei stand for the enzymes involved in fluxes	$\frac{\partial \Phi_i}{\partial Ei} > 0$, $i = 1, 4$.

Table 2.2 Genetic regulations in lipid metabolism

the fluxes are replaced by the corresponding functions of the enzymes given by Eqs.2.3,2.4,2.5,2.6, and of Eq.2.2.

Genetic variables in equilibrium ISDM Let us suppose that the observation time τ satisfies $\tau >> \tau_G$ where τ_G is the genetic time scale. Then, in order to obtain IDSM we shall eliminate the genetic variables from the equations :

$$F_1(A_2, PPAR) = F_2(A_2, LXR) = F_3(LXR, E1) = F_4(PPAR, E2) = F_5(PPAR, E3) = F_6(PPAR, E4) = 0 \tag{2.7}$$

Before this set of eliminations, the fluxes SYN, $OXI1,2$, $KOUT$ are functions of the metabolites and of $E1$, $E2$, $E3$, $E4$. After the eliminations, the fluxes $SYN_{equilib}$, $OXI1,2_{equilib}$, $KOUT_{equilib}$ are expressed as functions of metabolites only. This allow one to recover the signs of the genetic regulations that were justified intuitively in the previous subsection

Property 2
At equilibrium (that is, $\tau >> \tau_G$), genetic variables $PPAR, LXR, E1, E2, E3, E4$ can be expressed as functions of the metabolic variable A_2. The fluxes SYN, $OXI1,2$, $KOUT$ becomes functions of M, A_1, A_2, T only, denoted by $SYN_{equilib}$, $OXI1,2_{equilib}$, $KOUT_{equilib}$. The dependence of these functions on A_2 is the following.

$$\frac{\partial SYN_{equilib}}{\partial A_2} < 0, \qquad \frac{\partial OXI1,2_{equilib}}{\partial A_2} > 0, \qquad \frac{\partial KOUT_{equilib}}{\partial A_2} > 0.$$

Proof. The eliminations of genetic variables are possible in the neighborhood of any equilibrium because the matrix of partial derivatives $\frac{d\mathbf{F}}{d\mathbf{Y}}$ is triangular and its determinant is simply the product of the non-vanishing diagonal terms $\frac{\partial F_1}{\partial PPAR}$, $\frac{\partial F_2}{\partial LXR}$, $\frac{\partial F_3}{\partial E1}$, $\frac{\partial F_4}{\partial E2}$, $\frac{\partial F_5}{\partial E3}$, $\frac{\partial F_6}{\partial E4}$. Hence, one can express each genetic variable as a function of A_2, so that there exists five functions $PPAR_{equilib}(A_2)$, $LXR_{equilib}(A_2)$ $E1_{equilib}(A_2)$, $E2_{equilib}(A_2)$, $E3_{equilib}(A_2)$ such that

$$F_1(A_2, PPAR_{equilib}(A_2)) = F_2(A_2, LXR_{equilib}(A_2)) = F_3(LXR_{equilib}(A_2), E1_{equilib}(A_2)) = 0$$
$$F_4(PPAR_{equilib}(A_2), E2_{equilib}(A_2)) = F_5(PPAR_{equilib}(A_2), E3_{equilib}(A_2)) = F_6(PPAR_{equilib}(A_2), E4_{equilib}(A_2)) = 0$$

Let us denote $SYN_{equilib}(M,T) = \Phi_1(M,T,E1_{equilib}(A_2))$ the lipogenesis flux at equilibrium. Then $\frac{\partial SYN_{equilib}}{\partial A_2} = \frac{\partial \Phi_1}{\partial E1}\frac{dE1_{equilib}}{dA_2}$. By a derivation of the relation satisfied by F_3 and F_2, one gets:

$$\frac{\partial F_3}{\partial LXR}\frac{dLXR_{equilib}}{dA_2} + \frac{\partial F_3}{\partial E1}\frac{dE1_{equilib}}{dA_2} = 0 \quad \frac{\partial F_2}{\partial A_2} + \frac{\partial F_2}{\partial LXR}\frac{dLXR_{equilib}}{dA_2} = 0$$

Finally, the dependancy of the flux $SYN_{equilib}$ relatively to A_2 is given by:

$$\frac{\partial SYN_{equilib}}{\partial A_2} = -\frac{\partial \Phi_1}{\partial E1}\frac{\partial LXR_{equilib}}{\partial A_2}\frac{\partial F_3}{\partial LXR}\left[\frac{\partial F_3}{\partial E1}\right]^{-1} = \frac{\partial \Phi_1}{\partial E1}\frac{\partial F_3}{\partial LXR}\frac{\partial F_2}{\partial A_2}\left[\frac{\partial F_3}{\partial E1}\frac{\partial F_2}{\partial LXR}\right]^{-1}$$

The sign of each of the last fluxes shall be checked in Figure 2.2 so that $\frac{\partial SYN_{equilib}}{\partial A_2}$ is negative.
A similar proof gives the following:

$$\frac{\partial OXI2_{equilib}}{\partial A_2} = \frac{\partial \Phi_3}{\partial A_2} + \left[\frac{\partial F_1}{\partial A_2}\frac{\partial F_5}{\partial PPAR}\frac{\partial \Phi_3}{\partial E3}\right]\ \left[\frac{\partial F_1}{\partial PPAR}\frac{\partial F_5}{\partial E3}\right]^{-1} > 0$$

$$\frac{\partial OXI1_{equilib}}{\partial A_2} = \left[\frac{\partial F_1}{\partial A_2}\frac{\partial F_4}{\partial PPAR}\frac{\partial \Phi_2}{\partial E2}\right]\ \left[\frac{\partial F_1}{\partial PPAR}\frac{\partial F_4}{\partial E2}\right]^{-1} > 0$$

$$\frac{\partial KOUT_{equilib}}{\partial A_2} = \left[\frac{\partial F_1}{\partial A_2}\frac{\partial F_6}{\partial PPAR}\frac{\partial \Phi_4}{\partial E4}\right]\ \left[\frac{\partial F_1}{\partial PPAR}\frac{\partial F_6}{\partial E4}\right]^{-1} > 0$$

■

Genetic variables in quasi-stationary ISDM If $\tau_G > \tau >> \tau_M$, where τ_M is the metabolic time scale, then the equations 2.7 are no longer valid because genetic variables are not equilibrated. Therefore the relations between metabolites and the three genetically regulated fluxes are given by the Eqs.2.3,2.4,2.5,2.6 in which $Ei, i = 1,4$ are constants.

We are interested in the signs of the regulation arcs of the associated interaction graph that depend (among other) on the signs of the derivatives $\frac{\partial SYN}{\partial A_2}, \frac{\partial OXI}{\partial A_2}, \frac{\partial KOUT}{\partial A_2}$. The following relations say that for short observation times genetic regulations are not effective.

Property 3
At quasi-stationarity (that is, $\tau_G > \tau >> \tau_M$), genetic variables $PPAR, LXR, E1, E2, E3, E4$ are constant and are independent from metabolic variables M, A_1, A_2, T. The fluxes SYN, $OXI1,2$, $KOUT$ are functions of M, A_1, A_2, T only, denoted by $SYN_{quasi-sta}$, $OXI1,2_{quasi-sta}$, $KOUT_{quasi-sta}$. The dependence of these functions on A_2 is the following.

$$\frac{\partial SYN_{quasi-sta}}{\partial A_2} = 0, \qquad \frac{\partial OXI1_{quasi-sta}}{\partial A_2} = 0, \qquad \frac{\partial OXI2_{quasi-sta}}{\partial A_2} > 0, \qquad \frac{\partial KOUT_{quasi-sta}}{\partial A_2} = 0.$$

Equations of equilibrium and quasi-stationnary IDSM Let us denote $SYN_{IDSM}, OXI1,2_{IDSM}, KOUT_{IDSM}$ the fluxes obtained after elimination the genetic variables, that is $SYN_{equilib}$ or $SYN_{quasi-stat}$ depending on timescale. Then the IDSM of lipid metabolism corresponds to canceling the following fluxes on the metabolic variables M, T, A_1, A_2, G where each fluxes satisfies the derivative relations implicitly provided by regulations given in Table 2.3, and shown in Fig2.3, where three boxes where isolated.

Control coefficients and metabolic susceptibilities In metabolic control language, a control coefficient quantifies the dependency of a flux on an enzyme activity. In IDSM enzymes influences are implicit and we define the control coefficients as the absolute values of the partial derivatives of the elementary fluxes with respect to metabolites. According to this definition, the control coefficients are always positive. This choice is useful when the signs of the corresponding partial derivatives does not change when equilibrium changes; it allows identifying positive quantities and balances in the control equations.

Control coefficients correspond to various control mechanisms (genetic, hormonal, metabolic). Let us define the following *genetic control coefficients*:

$$R_{A2}^{O2} = \frac{\partial(OXI2_{IDSM}}{\partial A2} \tag{2.8}$$
$$R_{A2}^{O1} = \frac{\partial(OXI1_{IDSM}}{\partial A2} \tag{2.9}$$
$$R_{A2}^{OS1} = \frac{\partial(OXI1_{IDSM} - SYN_{IDSM})}{\partial A2} \tag{2.10}$$
$$R_{A2}^{KT} = \frac{\partial KOUT_{IDSM}}{\partial A2} \tag{2.11}$$

Let us also define the following *ATP control coefficients*:

$$T_{LP1} = -\frac{\partial AIN1}{\partial T} \tag{2.12}$$
$$T_{LP2} = -\frac{\partial AIN2}{\partial T} \tag{2.13}$$
$$T_{SO1} = \frac{\partial SYN_{IDSM} - OXI1_{IDSM}}{\partial T} \tag{2.14}$$
$$T_{O1} = -\frac{\partial OXI1_{IDSM}}{\partial T} \tag{2.15}$$
$$T_{O2} = -\frac{\partial OXI2_{IDSM}}{\partial T} \tag{2.16}$$
$$T_{KB} = -\frac{\partial KREBS}{\partial T} \tag{2.17}$$
$$T_{GL} = -\frac{\partial GLY}{\partial T} \tag{2.18}$$
$$T_{PDH} = -\frac{\partial PDH(\mathcal{M}_{P,box1})}{\partial T} \tag{2.19}$$
$$\tag{2.20}$$

Susceptibilities are self-control coefficients: they quantify how fluxes and rates of a metabolite depend on this metabolite. By definition, they are also chosen to be positive.

Let us define the following set of *metabolic susceptibilities*:

$$\chi_{A1}^{tot} = -\frac{\partial \Phi_{A1}}{\partial A1} = \frac{\partial(OXI1_{IDSM} - SYN_{IDSM} - AIN1)}{\partial A1} + \chi_{A1} \tag{2.21}$$
$$\chi_{A2}^{tot} = -\frac{\partial \Phi_{A2}}{\partial A2} = \frac{\partial(OXI2_{IDSM} - AIN2)}{\partial A2} + \chi_{A2} \tag{2.22}$$
$$\chi_{A1}^{O} = -\frac{\partial \Phi_{OXI1}}{\partial A1} \tag{2.23}$$
$$\chi_{A2}^{O} = -\frac{\partial \Phi_{OXI2}}{\partial A2} \tag{2.24}$$
$$\chi_{M}^{tot} = -\frac{\partial \Phi_{M}}{\partial M} = \frac{\partial(KREBS + KOUT_{IDSM} + SYN_{IDSM})}{\partial M} + \chi_{M} \tag{2.25}$$
$$\chi_{M}^{S} = -\frac{\partial \Phi_{SYN}}{\partial M} \tag{2.26}$$
$$\chi_{M}^{KB} = \frac{\partial \Phi_{KREBS}}{\partial M} \tag{2.27}$$

The following susceptibility ratios are smaller than one:

$$0 \leq \rho_{A1}^{O1} = \frac{\chi_{A1}^{OXI}}{\chi_{A1}^{tot}} \leq 1, 0 \leq \rho_{A2}^{O2} = \frac{\chi_{A2}^{OXI}}{\chi_{A2}^{tot}} \leq 1, 0 \leq \rho_{M}^{S} = \frac{\chi_{M}^{SYN}}{\chi_{M}^{tot}} \leq 1 \tag{2.28}$$

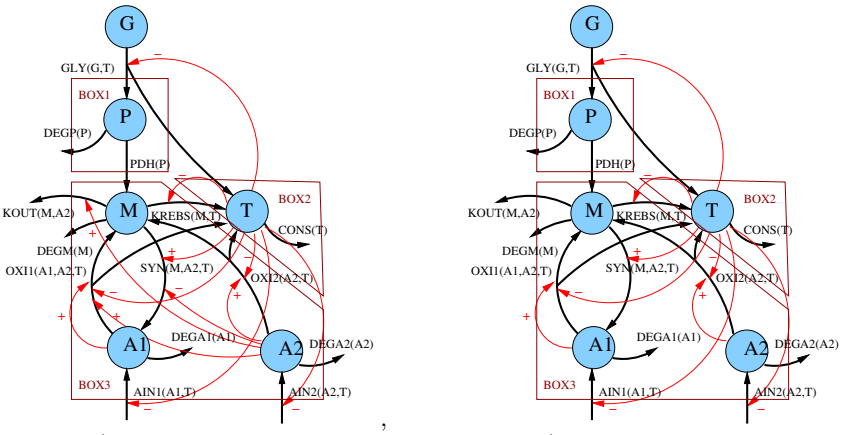
3 Study of equilibrium in lipid metabolism

3.1 Existence of an equilibrium

A theorem (Theorem 3 of Appendix) derived from the Brouwer fixed point theorem allows one to prove the existence of an equilibrium for the lipid metabolism.

Property 4
Supposing that

- *Oxidation does not function in the opposite direction (like a synthetic pathway) when there is no more acetyl-CoA or ATP.*



a) genetic timescale , b) metabolic timescale

Fig. 2.3 Fluxes, main metabolites and regulations of lipid metabolism if : a) $\tau >> \tau_G$ b) $\tau_G > \tau >> \tau_M$

- *If there is no more acetyl-coA, then the reaction PDH tries to produce it. The flux PDH is zero if* $P = 0$.
- *If the cell contains no unsaturated fatty acids, then unsaturated fatty acids enter the cell.*
- *Glycolysis, Oxidation, Krebs cycle, Lipogenesis, Ketone exits and Pyruvate dehydrogenase reaction are not reversible and have vanishing fluxes in absence of substrate.*
- *all fluxes saturate at high concentrations of metabolites.*
- *ATP consumption vanishes when ATP vanishes and increases at least linearly with ATP,*

Then, for any $G \geq 0$ *the system of equations 2.29,2.30,2.31,2.32,2.33, has at least a solution* $P \geq 0, A_1 \geq 0, A_2 \geq 0, M \geq 0, T \geq 0$.

Proof Because of the presence of degradation terms (and of the term CONS for T) the form of the system in Theorem3 of the Appendix. The conditions listed in the hypothesis are translated in the model as follows:

Condition 1
- $OXI1, 2 > 0$ *whenever* $M = 0$ *or* $T = 0$,
- $AIN1, 2(0, T) > 0$,
- $CONS(0) = 0$, $\frac{dCONS}{dT} > C > 0$,
- $GLY, KREBS, SYN_{IDSM}, KOUT_{IDSM}, PDH \geq 0$ and $OXI1_{IDSM}(0, A_2, T) = 0, OXI2_{IDSM}(0, T) = 0, KOUT_{IDSM}(0, A_2) = 0, SYN_{IDSM}(0, A_2, T) = 0, KREBS(0, T) = 0, PDH(0) = 0$,
- *all fluxes (except degradations and CONS) are bounded.*

These hypothesis appear to be sufficient to apply Theorem 3 of Appendix and to obtain the existence of a solution. A detailed proof is given in Appendix. ■

3.2 Unicity of equilibrium with Thomas rule?

Qualitative information on the system is summarized by the *interaction graph* associated to IDSM This graph gathers information related to the (direct or indirect) action of a metabolite on another metabolite.

Definition 2 *We call interaction graph associated to IDSM the signed oriented graph whose nodes are the metabolites. There is a regulation arc from* X_i *to* X_j *whenever* $\frac{\partial \Phi_j}{\partial X_i} \neq 0$ *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* $\frac{\partial \Phi_j}{\partial X_i}$.

Such an interaction graph for mixed networks is completely analogous to genetic interaction graphs as defined and studied by C.Soulé in [2]. All the properties proved for genetic regulation graphs are valid with no modification for the interaction graph of IDSM. One of these properties is the following, where a loop is defined as a closed circuit on the graph and the sign of the loop is the product of the signs of the arcs constituting the circuit.

Theorem 1 (Thomas rule, [1,3,4,2]) *If the interaction graph has no positive loop, then the system has a unique equilibrium.*

The signs of these partial derivatives for the lipid metabolism can be found by combining the Eqs.2.29, 2.30, 2.31, 2.33, 2.32 that give the expression of the fluxes as sums of elementary fluxes and the table in Fig.2.3 that gives the partial derivatives of elementary fluxes.

- Positive interaction: $\frac{\partial \Phi_P}{\partial G}$, $\frac{\partial \Phi_{A1}}{\partial M}$, $\frac{\partial \Phi_T}{\partial A_1}$,$\frac{\partial \Phi_T}{\partial A_2}$, $\frac{\partial \Phi_T}{\partial M}$, $\frac{\partial \Phi_T}{\partial G}$, $\frac{\partial \Phi_M}{\partial P}$, $\frac{\partial \Phi_M}{\partial A_1}$.
- Negative interaction: $\frac{\partial \Phi_P}{\partial T}$, $\frac{\partial \Phi_{A1}}{\partial A_2}$ (vanishing at quasistationarity), $\frac{\partial \Phi_P}{\partial P}$, $\frac{\partial \Phi_{A1}}{\partial A_1}$, $\frac{\partial \Phi_{A2}}{\partial A_2}$, $\frac{\partial \Phi_T}{\partial T}$, $\frac{\partial \Phi_M}{\partial M}$.
- Undetermined interaction:

$$
\begin{aligned}
(*) \quad & \frac{\partial \Phi_M}{\partial T} = T_{KB} - T_{SO1} - T_{O2} \\
(**) \quad & \frac{\partial \Phi_{A1}}{\partial T} = T_{SO1} - T_{LP1} \\
(***) \quad & \frac{\partial \Phi_{A2}}{\partial T} = T_{O2} - T_{LP2} \\
(****) \quad & \frac{\partial \Phi_M}{\partial A_2} = R_{A2}^{OS1} + R_{A2}^{O2} - R_{A2}^{KT} \text{(positive at quasi-stationarity)}
\end{aligned}
$$

The result of these computations is represented in Fig.3.1 for short and long observation times.Proceeding like this we realize that most of the regulation arcs have well defined and constant signs, but that there are some whose signs may change when equilibrium changes. The arcs whose signs are undetermined are denoted by stars in Figure 3.1.

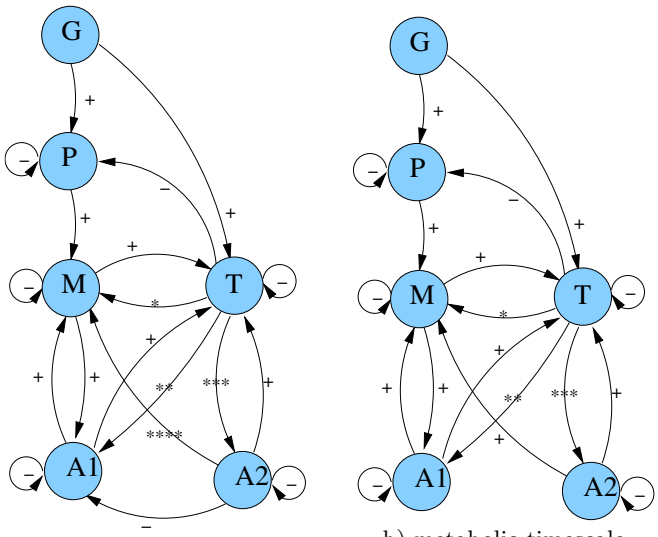


Fig. 3.1 Interaction graph. The stars mark regulation arcs whose sign may vary. The two situations correspond to different observation times τ: a) $\tau >> \tau_G$, b) $\tau_G > \tau >> \tau_M$.

Thomas rule provides a sufficient condition for the uniqueness of equilibrium and a necessary condition for multistationarity. The sufficient condition is the absence of positive loops in the interaction graph for all values of the node variables and external parameters. The necessary condition is the presence of such a positive loop for some values of the node variables and external parameters. Unfortunately, it appears that Thomas rule does not apply to the interaction graph of lipid metabolism: this sufficient condition is simply too strong; there is always a positive loop formed by the arcs connecting M and A_1.

Property 5
The interaction graph of the lipid metabolism model has a positive loop for any value of the node variables and of the external parameters. Hence Thomas criterion can not say whether equilibrium is unique or not.

3.3 Unicity of equilibrium with box equilibration

Since the Thomas sufficient condition is too strong, we shall find here milder sufficient conditions for uniqueness of equilibrium using a complete box equilibration of IDSM and our Property 1. This approach allows also a discussion of the equilibrium shifts under changes of the external conditions.

According to Property 1 a sufficient condition for uniqueness of equilibrium is to find a complete sequence of box equilibrations for which the systems of equations $\mathbf{\Phi}^{(i)}(\mathbf{X}^{(i)}, \mathbf{X}^{(e)}) = 0$ have an unique solution for the internal variables in all boxes.

In this section we shall not distinguish between the situations $\tau_G << \tau$ and $\tau_G > \tau >> \tau_M$. All the properties of this section are valid both for long and for short observation times. The short time situation corresponds to $\frac{\partial SYN}{\partial A_2} = 0, \frac{\partial OXI1}{\partial A_2} = 0$, $\frac{\partial KOUT}{\partial A_2} = 0$ and these identities do not affect the validity of any of the proofs below.

Let us consider the boxes as in Fig.2.3 and the sequence 1,3,2. This is a complete sequence because it exhausts all metabolites. The details of the box equilibration are given in Appendix. The main steps are the following:

– The equilibration equation for the internal variable P is:

$$\Phi_P(G, T, P) = 0 \tag{3.1}$$

This equation has an unique solution in P, which is a function of G, T: $P = \mathcal{M}_{P,box1}(G, T)$.

– The equilibration equation for the internal variables M,A_1,A_2 is:

$$\Phi_{A1}(M, A_1, A_2, T) = \Phi_{A2}(A_2, T) = \Phi_M(M, A_1, A_2, T, \mathcal{M}_{P,box1}(G, T)) = 0 \tag{3.2}$$

This equation has an unique solution $M = \mathcal{M}_{M,box1,3}(G, T)$, $A_1 = \mathcal{M}_{A1,box1,3}(G, T)$, $A_2 = \mathcal{M}_{A2,box1,3}(G, T)$.

– The equilibration equation for the internal variables T is:

$$\Phi_T(\mathcal{M}_{M,box1,3}(G, T), \mathcal{M}_{A1,box1,3}(G, T), \mathcal{M}_{A2,box1,3}(G, T), T, G) = 0 \tag{3.3}$$

We shall provide a sufficient condition for this equation to have a unique solution.

Theorem 2 *When $\alpha_{O1} >> \alpha_K$ (β-oxidation produces much more ATP than the Krebs cycle), a sufficient condition for Eqs.3.1,3.3,3.2 to have unique solutions and therefore for the uniqueness of the equilibrium of the lipid regulated metabolism is:*

$$\alpha_{O1}\chi^O_{A1}\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} + (\alpha_{O1}R^{O1}_{A2} + \alpha_{02}\chi^O_{A2})\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} < 0 \tag{3.4}$$

This condition is equivalent to

$$\begin{aligned}(T_{LP2} - T_{O2})\left\{\alpha_{O2}\rho^{O2}_{A2} + \alpha_{O1}\frac{R^{O1}_{A2}}{\chi^{tot}_{A2}} + \frac{\alpha_{O1}\chi^O_{A2}}{\chi^{tot}_{A1}(1-\rho^{O1}_{A1}\rho^S_M)}\left[\frac{R^{OS1}_{A2}(1-\rho^S_M) - R^{KT}_{A2}\rho^S_M}{\chi^{tot}_{A2}} + \rho^{O2}_{A2}\rho^S_M\right]\right\} + \\ + \left[T_{LP1} - (1-\rho^S_M)T_{SO1} - \rho^S_M(T_{KB} - T_{PDH} - T_{O2})\right]\frac{\alpha_{O1}\rho^{O1}_{A1}}{1-\rho^{O1}_{A1}\rho^S_M} > 0\end{aligned} \tag{3.5}$$

We call this the Strong lypolytic response condition.

Proof. See Appendix. ∎

Biological Interpretation of the strong lipolytic condition Under fasting, an energetic drop (decrease of T) triggers a vigorous release of stored fatty acids that enter the cell. Eq. 3.5 says that the arrival of fatty acids inside the cell is boosted at a larger extent than other fluxes. The result is an accumulation of fatty acids in the cell.

It is useful to notice that the validity of Eq. 3.5 relies mainly on metabolic control that is effective at short as well as at long observation times. If there is no genetic control, $R^{O1}_{A2} = R^{OS1}_{A2} = R^{KT}_{A2} = 0$ and the validity of Eq. 3.5 still relies on the comparison between T_{LP2} and T_{O2}, and between T_{LP1} and T_{SO1}, T_{O2}, T_{PDH}.

Property 6
If one observes experimentally that $\frac{d\mathcal{M}_{A1}}{dG} < 0$, $\frac{d\mathcal{M}_{A2}}{dG} < 0$ *and if* $\frac{d\mathcal{M}_T}{dG} > 0$, *where* $\mathcal{M}_{A1}, \mathcal{M}_{A2}$ *are values of* A_1, A_2 *at equilibrium or quasi-equilibrium, this necessarily means that* $\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} < 0$, $\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} < 0$ *and therefore (condition 3.4) the equilibrium is unique. This is valid for both long and short observation times.*

Proof See Appendix ∎

It was observed experimentally that the decrease of food input shifts the equilibrium from a state with small A, therefore functioning in lipogenesis mode to a state with big A functioning in oxidation mode. The poly-unsaturated fatty acids content seems thus to be a good indicator of the change of the metabolic functioning mode.

Corrolary 1
The model for genetically regulated lipid metabolism predicts that if under fasting the regulating and de novo synthesized fatty acids increase inside the cell, then one should expect uniqueness of equilibrium; within the model all metabolic changes will be shifts and not switches of equilibrium. Furthermore this is valid for changes that take place either rapidly or slowly.

It is rather natural to consider that the control coefficient $\frac{d\mathcal{M}_T}{dG}$ is positive, meaning that with more food the cell has more energy at its disposal, and reciprocally with less food the energy available decreases. The strong lypolitic condition is compatible with this condition.

Property 7
Let $\frac{d\mathcal{M}_T}{dG}$ *be the the value of* T *at equilibrium or quasi-equilibrium. One has:*

$$\frac{d\mathcal{M}_T}{dG} = \left[\alpha_G \frac{\partial GLY}{\partial G} + \alpha_K \chi_M^{KB} \frac{\partial \mathcal{M}_{M,box1,3}}{\partial G} + \alpha_{O1}\chi_{A1}^{O} \frac{\partial \mathcal{M}_{A1,box1,3}}{\partial G} + \alpha_{O2}\chi_{A2}^{O} \frac{\partial \mathcal{M}_{A2,box1,3}}{\partial G}\right] /$$
$$/ \left[\alpha_G T_{GL} + \alpha_K T_{KB} + \alpha_{O1} T_{O1} + \alpha_{O2} T_{O2} + \chi_T - \alpha_K \chi_M^{KB} \frac{\partial \mathcal{M}_{M,box1,3}}{\partial T} - \alpha_{O1}\chi_{A1}^{O} \frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} - \right.$$
$$\left. -(\alpha_{O2}\chi_{A2}^{O} + \alpha_{O1} R_{A2}^{O1}) \frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T}\right] \tag{3.6}$$

From Eq.3.6 it follows:

1. *If the strong lypolytic response condition (Eqs.3.4,3.5) is satisfied, then* $\frac{d\mathcal{M}_T}{dG} > 0$. *This is valid for both long and short observation times.*
2. *If* $\frac{R_{A2}^{O1}}{R_{A2}^{KT}} > max(\frac{1}{1-\rho_{A1}^{O1}}, \frac{\rho_M^S}{1-\rho_M^S})$ *is satisfied, then* $\left(\frac{d\mathcal{M}_T}{dG}\right)_{equilib} < \left(\frac{d\mathcal{M}_T}{dG}\right)_{quasi-sta}$ *meaning that the onset of genetic regulation buffers the variations of* T.
3. *If* A_1 *regulated its oxidation,* $\left(\frac{d\mathcal{M}_T}{dG}\right)_{equilib}$ *would be higher, meaning a less efficient buffering effect on* T.

Proof See Appendix. ∎

Hence, our this model predicts some quantitative differences between rapid and slow changes of the food input. For rapid changes genetic regulation is not effective. We may expect that even in absence of genetic regulation metabolism will respond to fasting by activating oxidation that has a buffering effect on ATP. In our model this is true because of the "minimal service" ensured by hormonal and metabolic regulation. The role of genetic regulation is to reinforce the buffering effect, by increasing the efficiency of the response. Interestingly, the fact that regulating fatty acids are not synthesized de novo has a positive effect on the buffering efficiency.

4 Discussion

If our model is true, and if the experimental observation that fatty acids increase on fasting is correct, then the lipid metabolism changes are continuous equilibrium shifts. A direct experimental check would be to monitor the metabolism changes as a function of time or better as a function of the external parameter (food) and show the absence of discontinuous jumps or hysteresis.

It is rather interesting that we have obtained this conclusion indirectly and only using qualitative knowledge on the behavior of the system.

The advantage of equilibrium changes over equilibrium shifts is a gain in flexibility and the possibility of fine tuning.

Mathematically, our model is interesting because it shows an example of interaction graph with positive loops that still has an unique equilibrium. In this situation, Thomas condition concerning the absence of positive regulation loops is too strong and we propose a weaker sufficient condition for uniqueness of the equilibrium.

We also introduce here a general framework for dealing with equilibria and equilibria changes in mixed metabolic/genetic networks. Our equilibration method is modular and can take into account the biological modularity of networks.

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Appendix: Shifts and Switches

The two types of equilibrium changes SHIFT and SWITCH can be mathematically described as follows:

Let $F = F_p(x)$, $x \in U \subset \mathbb{R}^n$, $p \in I \subset \mathbb{R}$, be a smooth 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 p in a subinterval $J \subset I$, the vector field F_p admits a singular (equilibrium) point, that is: there exist $x \in U$ such that $F_p(x) = 0$. We call the 0-level (equilibrium) curve:

$$\mathcal{L} = \{ (p, x) \in J \times U \, , \, F_p(x) = 0 \}.$$

$\mathcal{L}$ 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 $x = L(p)$. Equivalently it means that for each value of $p \in J$ there exist a *unique* equilibrium point,

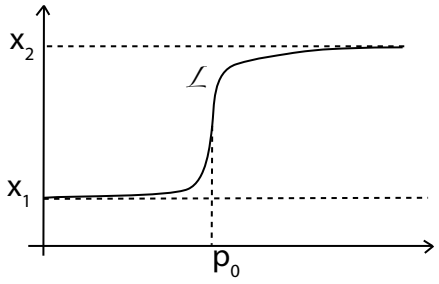

Fig. 4.1 A shift: the 0-level curve is a sigmoid

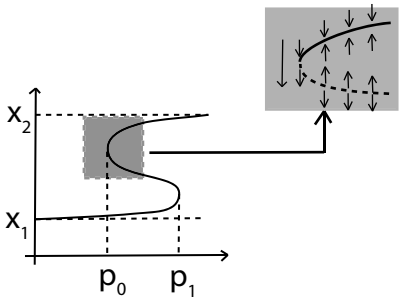

Fig. 4.2 A switch: the 0-level curve is not a graph. Around p_0 and p_1 a saddle-node bifurcation occurs.

- $\mathcal{L}$ is a sigmoid. That is there exist a threshold value p_0, an interval $]p_0-\delta, p_0+\delta[$, two equilibrium values x_1 and x_2 and $\epsilon > 0$ such that for $p < p_0 - \delta$, $|L(p) - x_1| < \epsilon$ and for $p > p_0 + \delta$, $|L(p) - x_2| < \epsilon$.

See an illustration in Picture 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 p_I, say $p_I < p_0 - \delta$, and increase smoothly the parameter up to a final value $p_F > p_0 + \delta$, we first observe an asymptotic state close to x_1 and a sudden "jump" to an asymptotic state close to x_2. We insist on that the apparent "jump" is due to the steepness of $\mathcal{L}$ at p_0 which induces a small δ: in reality there is no discontinuity. Now if we start with the value p_F and decrease smoothly the parameter, we note exactly the reverse: a jump at p_0 from an asymptotic state close to x_2 to an asymptotic state close to x_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 p_0 and p_1 for each of which the vector field has two singularities (equilibria) and for any $p \in]p_0, p_1[$, the vector field F_p has three singularities (one unstable and two stable). For $p < p_0$ or $p > p_1$ there is only one singularity (stable).
   - p_0 and 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 x_1 and x_2 and $\epsilon > 0$ such that for $p < p_0 - \delta$, $|L(p) - x_1| < \epsilon$ and for $p > p_1 + \delta$, $|L(p) - x_2| < \epsilon$.

See an illustration in Picture 4 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 p_I, say $p_I < p_0 - \delta$, and increase smoothly the parameter up to a final value $p_F > p_0 + \delta$, we first observe an asymptotic state close to x_1 and a sudden "jump" to an asymptotic state close to x_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 p_F and decrease smoothly the parameter, we note a jump at p_0 from an asymptotic state close to x_2 to an asymptotic state close to x_1. We do not have reversibility, because the jump occurs for different critical values of p. The experimental curves in both cases are depicted in Picture 4.

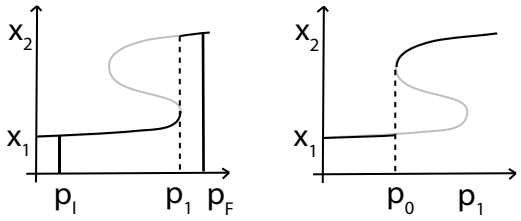

Fig. 4.3 Hysteresis effect: the experimental curves.

Appendix : Details of box equilibration, proof of Theorem 2

Box 1 equilibration The equilibration equation for the internal variable P is :

$$\Phi_P(G,T,P) = 0 \tag{4.1}$$

Property 8
Supposing

- *For* $P=0$, $\Phi_P = GLY(G,T) > 0$
- $PDH(P)$ *is bounded (saturation effect).*

The equation 4.1 has a unique solution in P, *denoted* $\mathcal{M}_{P,box1}(G,T)$. *Let* $PDH_{box1}(G,T) = PDH(\mathcal{M}_{P,box1}(G,T))$ *be the Pyruvate fluxes at box 1 equilibrium. Then* $\frac{\partial PDH_{box1}}{\partial G} > 0$ *and* $\frac{\partial PDH_{box1}}{\partial T} > 0$.

Proof From $\Phi_P = GLY(G,T) - \chi_P P - PDH(P)$ we get: $\frac{\partial \Phi_P}{\partial P} = -(\chi_P + \frac{dPDH}{dP}) = -\chi_P^{tot} < -\chi_P < 0$, so that $\Phi_P(G,T,P)$ is strictly monotonic, hence injective in P. The term $PDH(P)$ is bounded, therefore Φ_P is negative if P is big enough. For $P=0$, $\Phi_P = GLY(G,T) > 0$. Therefore a solution of $\Phi_P = 0$ exists and this solution is unique: there exists a unique function $\mathcal{M}_{P,box1}(G,T)$ such that $\Phi_P(G,T,\mathcal{M}_{P,box1}(G,T)) = 0$.

Differentiating this relation gives $\frac{\partial GLY}{\partial G} = \chi_P^{tot}\frac{\partial \mathcal{M}_{P,box1}}{\partial G}$ and $\frac{\partial GLY}{\partial T} = \chi_P^{tot}\frac{\partial \mathcal{M}_{P,box1}}{\partial T}$.

Let $PDH_{box1}(G,T) = PDH(\mathcal{M}_{P,box1}(G,T))$. Then $\frac{\partial PDH_{box1}}{\partial G} = \frac{dPDH}{dP}\frac{\partial \mathcal{M}_{P,box1}}{\partial G} = \frac{\frac{dPDH}{dP}\frac{\partial GLY}{\partial G}}{\chi_P^{tot}}$ that is positive from Table 2.3. Similarly $\frac{\partial PDH_{box1}}{\partial T} = \frac{\frac{dPDH}{dP}\frac{\partial GLY}{\partial T}}{\chi_P^{tot}} < 0$. ∎

Box 3 equilibration Let $\Phi_{M,box1}$ denote the flux of M after equilibration of box 1: $\Phi_{M,box1}(M,A,T,G) = \Phi_M(M,A,T,\mathcal{M}_P(G,T))$. The equilibration equations for the box 3 are:

$$\Phi_{M,box1}(M,A,T,G) = PDH_{box1}(T,G) + OXI1(A_1,A_2,T) + OXI2(A_2,T) - KREBS(M,T) - \\ -KOUT(M,A_2) - SYN(M,A_2,T) - \chi_M M = 0 \tag{4.2}$$

$$\Phi_{A1}(M,A_1,A_2,T) = SYN(M,A_2,T) - OXI1(A_1,A_2,T) + AIN1(A_1,T) - \chi_{A1}A_1 = 0 \tag{4.3}$$

$$\Phi_{A2}(M,A_2,T) = -OXI2(A_2,T) + AIN2(A_2,T) - \chi_{A2}A_2 = 0 \tag{4.4}$$

Property 9
Under the hypothesis of Property 4, the system of eqs.4.2,4.3,4.4 has a unique solution in (M,A_1,A_2). *This expresses* M, A_1, A_2 *as univoque functions of* G *and* T, *denoted by* $\mathcal{M}_{M,box1,3}(G,T)$ *and* $\mathcal{M}_{A1,2,box1,3}(G,T)$.

Proof Let us consider the mapping $(M,A_1,A_2) \to (\Phi_{M,box1},\Phi_{A1},\Phi_{A2})$. The Jacobian of this mapping is

$$J_{box1,3} = \begin{pmatrix} -\chi_M^{tot} & \chi_{A1}^O & \chi_{A2}^O + R_{A2}^{OS1} - R_{A2}^{KT} \\ \chi_M^S & -\chi_{A1}^{tot} & -R_{A2}^{OS1} \\ 0 & 0 & -\chi_{A2}^{tot} \end{pmatrix}$$

Using $\chi_M^S < \chi_M^{tot}, \chi_{A1}^O < \chi_{A1}^{tot}$ it can be easily checked that all principal minors of $-J_{box1,3}$ are positive.

The proof of the property follows from the Properties4, 1 and the following lemma (which is a direct consequence of Gale-Nikaido-Inada theorem [15]):

Lemma 1
If $(M, A_1, A_2) \to (\Phi_M, \Phi_{A1}, \Phi_{A2})$ 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 $\Phi_M = 0, \Phi_{A1} = 0, \Phi_{A2} = 0$ has a unique solution if a solution exists.

■

Box 2 equilibration We can use the property 9 to infer that M, A_1, A_2 are functions of T and G only, denoted by $\mathcal{M}_{M,box1,3}$, $\mathcal{M}_{A1,box1,3}$ and $\mathcal{M}_{A2,box1,3}$. Then $KREBS, OXI1, 2, \Phi_T$ become functions of G, T only.

For the box 2 the equilibration equation for the interior variable T is:

$$\Phi_{T,box1,3}(T,G) = \alpha_G GLY(G,T) + \alpha_K KREBS(\mathcal{M}_{M,box1,3}(G,T),T) + \alpha_{O1} OXI1(\mathcal{M}_{A1,box1,3}(G,T), \\ ,\mathcal{M}_{A2,box1,3}(G,T),T) + \alpha_{O2} OXI2(\mathcal{M}_{A2,box1,3}(G,T),T) - CONS(T) = 0 \quad (4.5)$$

Property 10
Eq.4.5 has a unique solution in T as soon as

$$\alpha_K \chi_M^{KB} \frac{\partial \mathcal{M}_{M,box1,3}}{\partial T} + \alpha_{01} \chi_{A1}^{O} \frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} + (\alpha_{O1} R_{A2}^{O1} + \alpha_{02} \chi_{A2}^{O}) \frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} < 0. \quad (4.6)$$

Proof The existence of the solution follows from $CONS \to \infty$ while $GLY, KREBS, OXI1, OXI2$ are bounded when $T \to \infty$, hence $\Phi_{T,box1,3}$ is negative for T sufficiently big, and from $GLY, KREBS, OXI1, OXI2 > 0$, $CONS = 0$ hence $\Phi_{T,box1,3} > 0$ when $T = 0$. Note that the existence of a solution follows also from Properties4, 1.

Eq. 4.5 implies:

$$\frac{\partial \Phi_{T,box1,3}}{\partial T} = \alpha_G \frac{\partial GLY}{\partial T} + \alpha_K (\chi_M^{KB} \frac{\partial \mathcal{M}_{M,box1,3}}{\partial T} - T_{KB}) + \alpha_{O1} (\chi_{A1}^{O} \frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} + R_{A2}^{O1} \frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} - T_{O1}) + \\ + \alpha_{O2} (\chi_{A2}^{O} \frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} - T_{O2}) - \frac{\partial CONS}{\partial T} \quad (4.7)$$

Using Table 2.3, Eq.4.7, and Condition 4.6 it follows that $\frac{\partial \Phi_{T,box1,3}}{\partial T} < 0$, hence that Eq.4.5 has a unique solution in T as soon as a solution exists, which was stated before. ■

We can now give a proof of Theorem2.

Proof of Theorem 2 When $\alpha_{O1} >> \alpha_K$, and supposing that $\chi_M^{KB} \frac{\partial \mathcal{M}_{M,box1,3}}{\partial T}$, $\chi_{A1}^{O} \frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T}$ have similar orders of magnitude, one can safely reduce Eq.4.6 to Eq.3.4.

The partial derivatives with respect to T of the metabolites after equilibration of the boxes 1,3 can be obtained using the inverse of the Jacobian $J_{box1,3}$:.

$$\frac{\partial}{\partial T} \begin{pmatrix} \mathcal{M}_{M,box1,3} \\ \mathcal{M}_{A1,box1,3} \\ \mathcal{M}_{A2,box1,3} \end{pmatrix} = -J_{box1,3}^{-1} \frac{\partial}{\partial T} \begin{pmatrix} \Phi_{M,box1} \\ \Phi_{A1} \\ \Phi_{A2} \end{pmatrix} = -J_{box1,3}^{-1} \begin{pmatrix} -T_{PDH} - T_{O2} + T_{KB} - T_{SO1} \\ T_{SO1} - T_{LP1} \\ T_{O2} - T_{LP2} \end{pmatrix}$$

It follows:

$$\frac{\partial \mathcal{M}_{M,box1,3}}{\partial T} = -\frac{(T_{LP2} - T_{O2}) \left[\frac{R_{A2}^{OS1}(1-\rho_{A1}^{O1}) - R_{A2}^{KT}}{\chi_{A2}^{tot}} + \rho_{A2}^{O2} \right] + T_{SO1}(1-\rho_{A1}^{O1}) + T_{LP1}\rho_{A1}^{O1} + T_{PDH} + T_{O2} - T_{KB}}{\chi_M^{tot}(1-\rho_{A1}^{O1}\rho_M^S)} \quad (4.8)$$

$$\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} = -\frac{(T_{LP2} - T_{O2}) \left[\frac{R_{A2}^{OS1}(1-\rho_M^S) - R_{A2}^{KT}\rho_M^S}{\chi_{A2}^{tot}} + \rho_{A2}^{O2}\rho_M^S \right] + T_{LP1} - (1-\rho_M^S)T_{SO1} + \rho_M^S(T_{PDH} + T_{O2} - T_{KB})}{\chi_{A1}^{tot}(1-\rho_{A1}^{O1}\rho_M^S)} \quad (4.9)$$

$$\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} = -\frac{T_{LP2} - T_{O2}}{\chi_{A2}^{tot}} \quad (4.10)$$

Thus, the conditions 3.4,3.5 are equivalent. ■

Property 11
If $\frac{\partial \mathcal{M}_{A,box1,3}}{\partial T} < 0$ *is satisfied, then* $\frac{d\mathcal{M}_T}{dG} > 0$. *This is valid for both long and short observation times.*

Proof

The partial derivatives with respect to G of the metabolites after equilibration of the boxes 1,3 can be obtained from:

$$\frac{\partial}{\partial G}\begin{pmatrix}\mathcal{M}_{M,box1,3}\\ \mathcal{M}_{A1,box1,3}\\ \mathcal{M}_{A2,box1,3}\end{pmatrix} = -J^{-1}_{box1,3}\frac{\partial}{\partial G}\begin{pmatrix}\Phi_{M,box1}\\ \Phi_{A1}\\ \Phi_{A2}\end{pmatrix} = -J^{-1}_{box1,3}\begin{pmatrix}\frac{\partial PDH_{box1,3}}{\partial G}\\ 0\\ 0\end{pmatrix}$$

It follows:

$$\frac{\partial \mathcal{M}_{M,box1,3}}{\partial G} = \frac{\partial PDH_{box1,3}}{\partial G}\frac{\chi^{tot}_{A1}}{\chi^{tot}_{A1}\chi^{tot}_{M}-\chi^{O}_{A1}\chi^{S}_{M}} > 0 \tag{4.11}$$

$$\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial G} = \frac{\partial PDH_{box1,3}}{\partial G}\frac{\chi^{S}_{M}}{\chi^{tot}_{A1}\chi^{tot}_{M}-\chi^{O}_{A1}\chi^{S}_{M}} > 0 \tag{4.12}$$

$$\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial G} = 0 \tag{4.13}$$

From Eq. 4.5 it follows:

$$\frac{d\mathcal{M}_T}{dG} = -\frac{\partial \Phi_{T,box1,3}}{\partial G}/\frac{\partial \Phi_{T,box1,3}}{\partial T} = \alpha_G\frac{\partial GLY}{\partial G} + \alpha_K\chi^{KB}_M\frac{\partial \mathcal{M}_{M,box1,3}}{\partial G} + \alpha_{O1}\chi^{O}_{A1}\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial G} + \alpha_{O2}\chi^{O}_{A2}\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial G} \;/$$

$$/\;\; \alpha_G T_{GL} + \alpha_K T_{KB} + \alpha_{O1}T_{O1} + \alpha_{O2}T_{O2} + \chi_T - \alpha_K\chi^{KB}_M\frac{\partial \mathcal{M}_{M,box1,3}}{\partial T} - \alpha_{O1}\chi^{O}_{A1}\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial T} -$$

$$-(\alpha_{O2}\chi^{O}_{A2} + \alpha_{O1}R^{O1}_{A2})\frac{\partial \mathcal{M}_{A2,box1,3}}{\partial T} \tag{4.14}$$

Under the condition 3.4 from Eqs.4.11,4.14 it follows $\frac{d\mathcal{M}_T}{dG} > 0$. ■

Property 12
If $\frac{R^{O1}_{A2}}{R^{KT}_{A2}} > max(\frac{1}{1-\rho^{O1}_{A1}}, \frac{\rho^{S}_{M}}{1-\rho^{S}_{M}})$ *is satisfied, then* $\left(\frac{d\mathcal{M}_T}{dG}\right)_{equilib} > \left(\frac{d\mathcal{M}_T}{dG}\right)_{quasi-sta}$. *If* A_1 *regulated its oxidation,* $\left(\frac{d\mathcal{M}_T}{dG}\right)_{equilib}$ *would be higher.*

Proof The difference between equilibrium and quasi-stationarity occurs at two levels. For quasistationarity $R^{OS1}_{A2} = R^{O1}_{A2} = R^{KT}_{A2} = 0$ and the values of $\chi^{O}_{A2}, \rho^{O2}_{A2}$ are decreased with respect to equilibrium (because of the absence at quasistationarity of genetic regulation of A_2 on its own oxidation). The conclusion of the property is straightforward from Eqs.4.14,4.8,4.11. Genetic regulation increases the absolute values of $\frac{\partial \mathcal{M}_{M,box1,3}}{\partial G}$, $\frac{\partial \mathcal{M}_{A1,box1,3}}{\partial G}$, $\frac{\partial \Phi_{T,box1,3}}{\partial T}$, and does not change $\frac{\partial \Phi_{T,box1,3}}{\partial G}$. One can notice that if A_1 regulated its oxidation, χ^{O}_{A1} would increase and the absolute value of $\frac{\partial \Phi_{T,box1,3}}{\partial G}$ would increase as well, leading to a an increase of $\left(\frac{d\mathcal{M}_T}{dG}\right)_{equilib}$.

■

We can give now the:

Proof of Property 6

The value of $A_i, i = 1,2$ at equilibrium or quasiequilibrium is a function of G, $\mathcal{M}_{Ai} = \mathcal{M}_{Ai,box1,3}(G, \mathcal{M}_T(G))$. The change in $A_i, i = 1,2$ induced by a change in G is given by :

$$\frac{d\mathcal{M}_{A_i}}{dG} = \frac{\partial \mathcal{M}_{Ai,box1,3}}{\partial G} + \frac{\partial \mathcal{M}_{Ai,box1,3}}{\partial T}\frac{d\mathcal{M}_T}{dG} \tag{4.15}$$

If one observes experimentally that $\frac{d\mathcal{M}_{Ai}}{dG} < 0$ and if $\frac{d\mathcal{M}_T}{dG}$, then from Eq.4.15 this necessarily means that $\frac{\partial \mathcal{M}_{Ai,box1,3}}{\partial T}$ because $\frac{\partial \mathcal{M}_{Ai,box1,3}}{\partial G} > 0$ (Eq.4.11). ■

Appendix : proof of Proposition 4

The proof uses the following more general theorem:

Theorem 3 *Let* $\mathbf{\Phi}(\mathbf{X}) = \mathbf{G}(\mathbf{X}) - \mathbf{\Lambda}(\mathbf{X})$ *be a vector field on* $\mathbb{R}^n_+$ *(*$\mathbb{R}^n_+$ *represents all the vectors of* $\mathbb{R}^n$ *having positive coordinates) such that :*

i) $\mathbf{G}$ *is differentiable, bounded and satisfies* $G_i(\ldots, X_i = 0, \ldots) > 0$.

ii) $\mathbf{\Lambda} = (\Lambda_1(X_1), \ldots, \Lambda_n(X_n))$ *where* Λ_i *are differentiable and satisfy* $\Lambda_i(0) = 0$ *and* $\frac{d\Lambda_i}{dX_i} \geq C_i > 0$.

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

Proof of Theorem3 From ii) it follows that $\mathbf{\Lambda}$ is invertible and $\mathbf{\Lambda}^{-1}\mathbf{G}$is bounded. Then the existence of a zero of $\mathbf{\Phi}$ is equivalent to the existence of a fixed point of the bounded function $\mathbf{\Lambda}^{-1}\mathbf{G}$. We can extend by symmetry $\mathbf{\Lambda}^{-1}\mathbf{G}$ to a bounded continuous function on $\mathbb{R}^n$. The Brouwer fixed point theorem [9] implies the existence of a fixed point of this function in $\mathbb{R}^n \cap \mathcal{K}$, where $\mathcal{K}$ is a compact within the bounds of $\mathbf{\Lambda}^{-1}\mathbf{G}$ (but not necessarily in $\mathbb{R}^n_+$). From i) it follows that $0 \leq G_i < G_o$ on $\Delta = \{\mathbf{x} | 0 \leq x_i \leq \epsilon_i, i = 1, n\}$. For large enough constants C_i in ii) it follows that the compact Δ is invariant under $\mathbf{\Lambda}^{-1}\mathbf{G}$. The Brouwer fixed point theorem can be applied again to show that $\mathbf{\Phi}$ has zeros in Δ, hence in $\mathbb{R}^n_+$, for large enough C_i. The rest of the proof is a continuity argument. One should notice that $\mathbf{\Phi}(C)$ has zeros in $\mathbb{R}^n_+ \cap \mathcal{K}$ for large C, has zeros in $\mathbb{R}^n \cap \mathcal{K}$ for any C. From i) and ii) it follows that no zeros can be found on the boundaries $x_i = 0$. It is enough to construct the Brouwer mapping degree [9], and a homotopy between $\mathbf{\Phi}(C)$ for a large and an arbitrary value of C to finish the proof.

■

Using Conditions1 it is easy to check that

$$\mathbf{G} = \begin{pmatrix} GLY - PDH \\ SYN_{IDSM} - OXI1_{IDSM} + AIN1 \\ -OXI2_{IDSM} + AIN2 \\ \alpha_G GLY + \alpha_K KREBS + \alpha_{O1} OXI1_{IDSM} + \alpha_{O2} OXI2_{IDSM} \\ PDH + OXI1_{IDSM} + OXI2_{IDSM} - KREBS - KOUT_{IDSM} - SYN_{IDSM} \end{pmatrix}$$

and $\mathbf{\Lambda} = \begin{pmatrix} DEGP \\ DEGA1 \\ DEGA2 \\ CONS \\ DEGM \end{pmatrix}$

fullfil the hypothesis of the theorem.

Flux	Symbol	Effect	Equilibrium regulations $\tau >> \tau_G$	Quasi-stationary regulations $\tau_G > \tau >> \tau_M$
Glycolysis	$GLY(G,T)$	consumes G produces P,T	$\frac{\partial GLY}{\partial G} > 0$ (metabolic) $\frac{\partial GLY}{\partial T} < 0$ (metabolic,hormonal)	$\frac{\partial GLY}{\partial G} > 0$ $\frac{\partial GLY}{\partial T} < 0$
Pyruvate dehydrogenase reaction	$PDH(P)$	consumes P produces M	$\frac{\partial PDH}{\partial P} > 0$ (metabolic)	$\frac{\partial PDH}{\partial P} > 0$
Krebs cycle	$KREBS(M,T)$	consumes M produces T	$\frac{\partial KREBS}{\partial M} > 0$ (metabolic) $\frac{\partial KREBS}{\partial T} < 0$ (metabolic)	$\frac{\partial KREBS}{\partial M} > 0$ $\frac{\partial KREBS}{\partial T} < 0$
ATP consumption	$CONS(T)$	consumes T	$\frac{\partial CONS}{\partial T} > 0$ (metabolic)	$\frac{\partial CONS}{\partial T} > 0$
Fatty acids A_1 enter/exit	$AIN1(A_1,T)$	produces or consumes A_1	$\frac{\partial AIN1}{\partial A_1} < 0$ (metabolic) $\frac{\partial AIN1}{\partial T} < 0$ (hormonal)	$\frac{\partial AIN1}{\partial A_1} < 0$ $\frac{\partial AIN1}{\partial T} < 0$
Fatty acids A_2 enter/exit	$AIN2(A_2,T)$	produces or consumes A_2	$\frac{\partial AIN2}{\partial A_2} < 0$ (metabolic) $\frac{\partial AIN2}{\partial T} < 0$ (hormonal)	$\frac{\partial AIN2}{\partial A_2} < 0$ $\frac{\partial AIN2}{\partial T} < 0$
Lipogenesis	$SYN_{IDSM}(M,A_2,T)$	consumes M produces A_1	$\frac{\partial SYN_{IDSM}}{\partial M} > 0$ (metabolic) $\frac{\partial SYN_{IDSM}}{\partial T} > 0$ (hormonal,metabolic) $\frac{\partial SYN_{IDSM}}{\partial A_2} < 0$ (genetic)	$\frac{\partial SYN_{IDSM}}{\partial M} > 0$ $\frac{\partial SYN_{IDSM}}{\partial T} > 0$ $\frac{\partial SYN_{IDSM}}{\partial A_2} = 0$
$\beta-$oxidation of A_1	$OXI1_{IDSM}(A_1,A_2,T)$	consumes A_1 produces M,T	$\frac{\partial OXI1_{IDSM}}{\partial A_1} > 0$ (metabolic) $\frac{\partial OXI1_{IDSM}}{\partial T} < 0$ (metabolic,hormonal) $\frac{\partial OXI1_{IDSM}}{\partial A_2} < 0$ (genetic)	$\frac{\partial OXI1_{IDSM}}{\partial A_1} > 0$ $\frac{\partial OXI1_{IDSM}}{\partial T} < 0$ $\frac{\partial OXI1_{IDSM}}{\partial A_2} = 0$
$\beta-$oxidation of A_2	$OXI2_{IDSM}(A_2,T)$	consumes A_2 produces M,T	$\frac{\partial OXI2_{IDSM}}{\partial A_2} > 0$ (metabolic,genetic) $\frac{\partial OXI2_{IDSM}}{\partial T} < 0$ (metabolic,hormonal)	$\frac{\partial OXI2_{IDSM}}{\partial A_2} > 0$ $\frac{\partial OXI2_{IDSM}}{\partial T} < 0$
Ketonic body exit	$KOUT_{IDSM}(M,A_2)$	consumes M	$\frac{\partial KOUT_{IDSM}}{\partial M} > 0$ (metabolic) $\frac{\partial KOUT_{IDSM}}{\partial A_2} > 0$ (genetic)	$\frac{\partial KOUT_{IDSM}}{\partial M} > 0$ $\frac{\partial KOUT_{IDSM}}{\partial A_2} = 0$
A_1 degradation	$DEGA1(A_1)$ $= \chi_{A1}A_1$	consumes A_1	$\chi_{A1} = \frac{\partial DEGA1}{\partial A_1} > 0$	$\chi_{A1} =$ $= \frac{\partial DEGA1}{\partial A_1} > 0$
A_2 degradation	$DEGA2(A_2)$ $= \chi_{A2}A_2$	consumes A_2	$\chi_{A2} = \frac{\partial DEGA2}{\partial A_2} > 0$	$\chi_{A2} =$ $= \frac{\partial DEGA2}{\partial A_2} > 0$
M degradation	$DEGM(M)$ $= \chi_M M$	consumes M	$\chi_M = \frac{\partial DEGM}{\partial M} > 0$	$\chi_M =$ $= \frac{\partial DEGM}{\partial M} > 0$
P degradation	$DEGP(P)$ $= \chi_P P$	consumes P	$\chi_P = \frac{\partial DEGP}{\partial P} > 0$	$\chi_P = \frac{\partial DEGP}{\partial P} > 0$

$$\Phi_P(G,T,P) = GLY(G,T) - DEGP(P) - PDH(P) = 0 \tag{2.29}$$

$$\Phi_{A1}(M,A_1,A_2,T) = SYN_{IDSM}(M,A_2,T) - OXI1_{IDSM}(A_1,A_2,T) + AIN1(A_1,T) - DEGA1(A_1) = 0 \tag{2.30}$$

$$\Phi_{A2}(M,A_2,T) = -OXI2_{IDSM}(A_2,T) + AIN2(A_2,T) - DEGA2(A_2) = 0 \tag{2.31}$$

$$\Phi_T(M,A_1,A_2,T,G) = \alpha_G GLY(G,T) + \alpha_K KREBS(M,T) + \alpha_{O1}OXI1_{IDSM}(A_1,A_2,T) + \alpha_{O2}OXI2_{IDSM}(A_2,T) - CONS(T) = 0 \tag{2.32}$$

$$\Phi_M(M,A_1,A_2,T,P) = PDH(P) + OXI1_{IDSM}(A_1,A_2,T) + OXI2_{IDSM}(A_2,T) - KREBS(M,T) - KOUT_{IDSM}(M,A_2) - SYN_{IDSM}(M,A_2,T) - DEGM(M) = 0 \tag{2.33}$$

Table 2.3 IDSM associated with lipid metabolism, including the implicit regulations relations between variables.