Abstract qualitative model for the genetically regulated lipid metabolism

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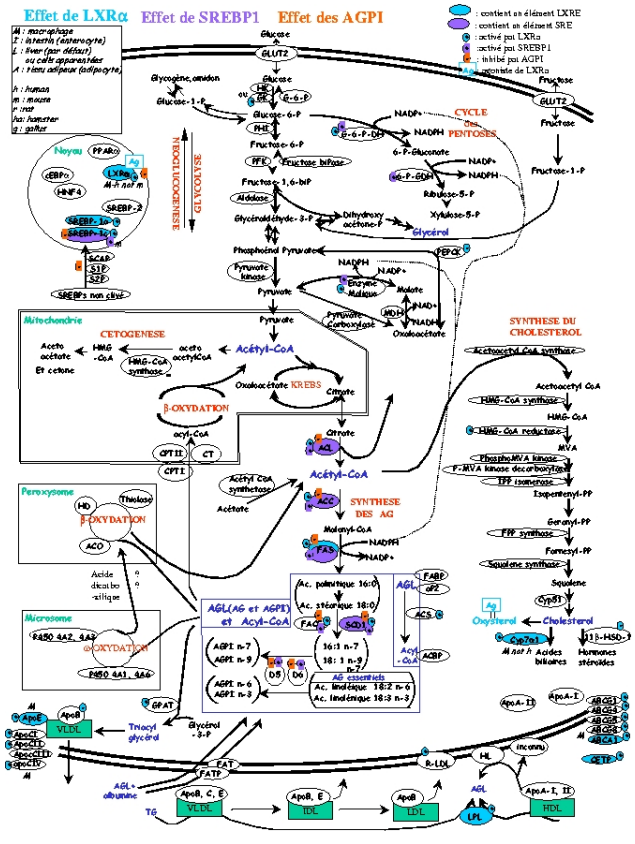
Lipid metabolism in chicken/mouse liver cells



- Two antagonistic functioning modes
  - synthesis and storage produce reserves (induced by normal feeding)
  - lipolysis and oxidation burn reserves and produce energy (induced by a lack of food)
- Complex regulations
  - intrinsic regulations related to metabolic biochemistry
  - genetic regulations of nuclear receptors on enzymes of metabolic pathways
  - action of fatty acids (metabolite) on genes controlling their metabolism

Mixed regulation network whose nodes are metabolites as well as genetic variables

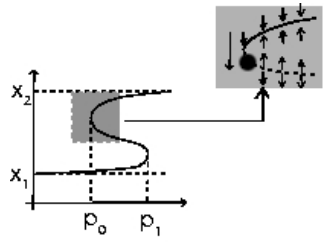
Modelling mixed regulation networks ?

- prokaryotes (lactose operon in E.Coli)
- eukariotes
  - metabolic pathways studied separately from their genetic regulation : problems on long timescales
  - Petri networks : glycolysis [Mastuno et al., Chaouyia et al]
  - Piecewise differential sytems : nutritional stress in E. Coli [Ropers and al.]
  - Simulation of differential models [Chabrier and al.]
  - Hybrid models [Langley et al., King et al.]

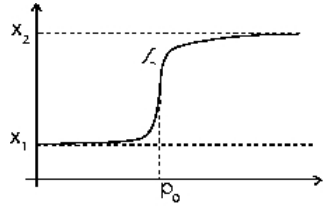
Three main questions (at the moment)

Adapting to external changes ?

- Bistability
  - Historical example : E.Coli bacterium lactose operon
  - the change in lactose induces an equilibrium switch : jump from one attractor to another.
  - efficient in saving resources (enzymes) [produced only on demand]
  - less flexible [binary type response] : minimal threshold stimulus needed to act ; tuning is not possible



- Equilibrium shift
  - uniqueness condition is fulfilled
  - no jump between attractors ; smooth, gradual changes.



Question 1 : *Do the regulations (metabolic, genetic, hormonal) of lipid metabolism produce multistationarity or an unique equilibrium ?*

Answer : Unique equilibrium under reasonable biological assumptions.

Interactions between metabolic and genetic pathways ?

Action of PUFA's ?

- only a special class (polyunsaturated fatty acids denoted by PUFA) interfere with genes
- PUFA are not synthetized by the organism : produced from essential fatty acids taken from the diet
- Control of metabolism (their own oxidation and synthesis, and oxidation of de novo fatty acids)
- Interactions with nuclear receptors regulating the transcription of genes coding for enzymes involved in the corresponding pathways.

Question 2 : *quantify the effect of PUFA on the lipid metabolism ?*

Answer : PPAR knock-out reduces energy buffering and increases PUFA entering during fasting.

Interactions between metabolic and genetic pathways ?

Role of genetic machinery ?

- Long timescales : Genetic regulation becomes effective only when transcriptional machinery is activated and processed
- On short timescales genetic variables can be considered to be constant
- Changes of nutritional conditions ask for genetic readjustments [fasting demands a shift from lipogenetic to lipolytic functioning modes]
- Genetic regulation brings slow but larger changes that push the shift further.

Question 3 : *Differences between fast and slow response of the system ?*

Answer : Genetic regulations reinforce the energy buffering effect.

Method

1. Construction of a mixed differential model
   - simplified description : 12 main variables
   - includes the energy available to the cell [variable for ATP concentration]
   - do not use explicit forms for flux and regulations : only use their variations with respect to the variables
2. Study of equilibria : sufficient condition for the uniqueness of equilibrium
3. Qualitative validation and prediction
   - effect of suppressing some genetic regulation,
   - role of genetic regulation for energy recovering at fasting.
4. Generic explicit model : numerical simulations
5. Mathematical framework : successive elimination of variables to compare equilibria

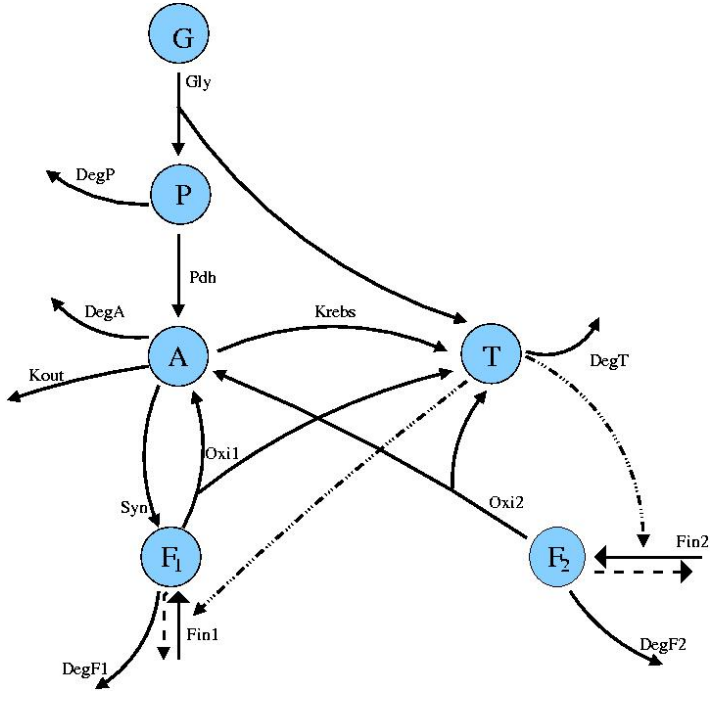
Construction of the model

Characteristics of the model

- Integrative model
  - main processes of carbohydrate and lipid metabolism in liver
  - various regulations (metabolic, genetic, hormonal)
- Not explicitly distributed (no space information is taken into account)
- Low complexity abstraction
  - basic features of metabolism in the main nutritional states
  - complex metabolic chains of reactions modeled as a single global reaction
- Keep the model as qualitative as possible
  - no specific numerical values of kinetic constants
  - no specific forms of the functions relating fluxes to concentrations
  - sufficient qualitative conditions chosen as biologically significant as possible

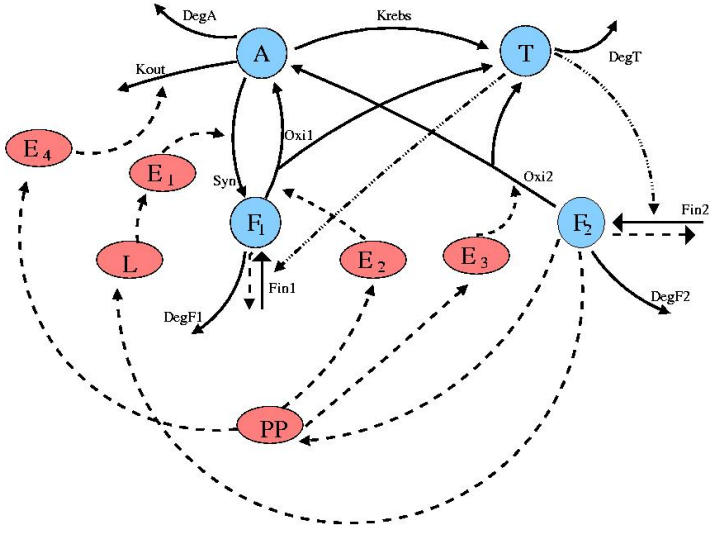
Metabolic variables and primitive fluxes

- Metabolic variables
  - Acetyl-CoA $[A]$ (mitochondria)
  - De novo synthesized fatty acids $[F_1]$ (produced from Acetyl-CoA)
  - Exogenous PUFA $[F_2]$ (brought by diet)
  - Energy (ATP) $[T]$ (energy in the cell)
  - Pyruvate $[P]$ (end of glycolysis)
- Parameter : glucose concentration $[G]$ (representing food)
- Primitive fluxes
  - lipid metabolism : Glycolysis, Pdh, Krebs cycle, lipogenesis, $\beta-$oxidation
  - ketone bodies exit transfers energy to the outside
  - Outtake/intake flux allows F_1 and F_2 to exit or enter the liver cell.
  - Degradation of metabolites is needed on the genetic timescale.
  - ATP consumption (energy consumed for living).



Genetic variables

- Nuclear receptors
  - PP : active form of the nuclear receptor PPAR (heterodimer with RXR),
  - L : active form of the nuclear receptor LXR (heterodimer with RXR)
- Metabolic fluc enzyme
  - E_1 : abstract enzyme modelling the set of enzymes involved in de novo fatty acids synthesis
  - E_2 : abstract enzyme modelling de novo acids oxidation,
  - E_3 : abstract enzyme modelling PUFA oxidation
  - E_4 : abstract enzyme modelling etone bodies exit
- Genetic control
  - LXR and PPAR control the production of the abstract enzymes E_i
  - PUFA control the production of active LXR and PPAR.

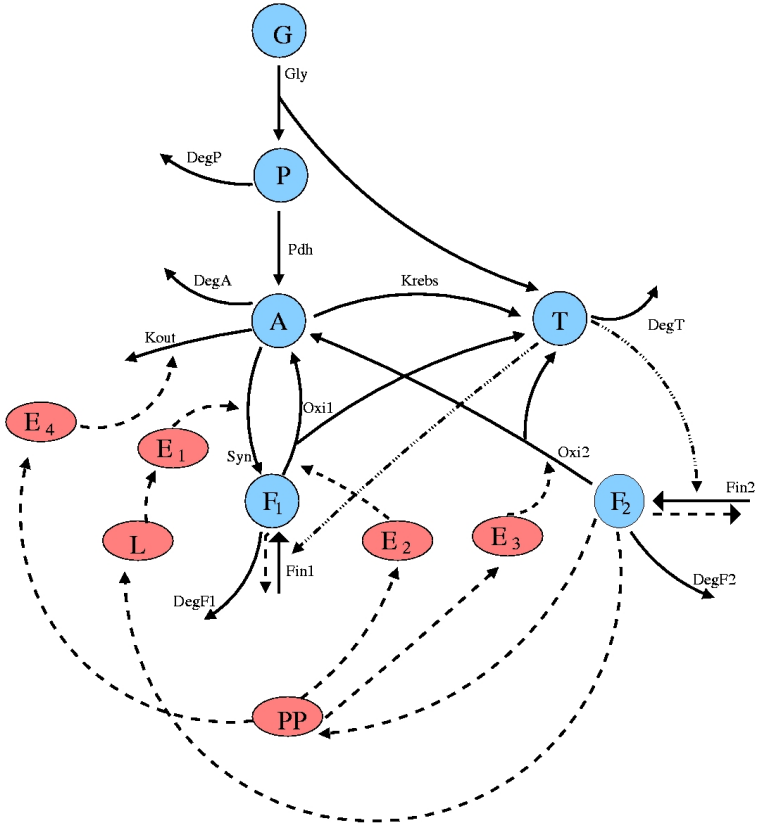


Main products and fluxes

Variable (Concentration)	Symbol	$\frac{d\ \text{product}}{dt}$
Acetyl Co-A	A	Φ_A
Pyruvate	P	Φ_P
De novo synthesized fatty acids	F_1	Φ_{F_1}
PUFA	F_2	Φ_{F_2}
Energy ATP	T	Φ_T
Active form of PPAR	PP	Ψ_1
Active form of LXR	L	Ψ_2
Enzymes of de novo fatty acids synthesis	E_1	Ψ_3
Enzymes of de novo fatty acids oxidation	E_2	Ψ_4
Enzymes of PUFA oxidation	E_3	Ψ_5
Enzymes of Ketone body exit	E_4	Ψ_6

Parameter	Symbol
Glucose	G

Primitive flux	Symbol
Glycolysis	Gly
Pyruvate dehydrogenase reaction	Pdh
Krebs cycle	Krebs
Ketone bodies exit	Kout
Lipogenesis	Syn
$\beta-$oxidation of de novo fatty acids	Oxi1
$\beta-$oxidation of PUFA	Oxi2
De Novo fatty acids intake/outake	Fin1
PUFA fatty acids intake/outake	Fin2
ATP consumption	DegT
Degradation of a metabolite V ($V = P, A, F_1, F_2$)	DegV



Differential model ?

Express the flux of each variable in terms of primitive fluxes

- Production of metabolic variables : sum of primitive fluxes that produce or consume the metabolite.
- Linear degradation reactions (exept for ATP)
- No details on variations of the genetic variables [unknown mechanisms]

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

Remark : *Only fluxes are modelled here. No regulation information is provided yet. What is the sign of each* $\frac{\partial\, flux}{\partial\, variable}$ *?*

Abstract differential model for regulated lipid metabolism

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

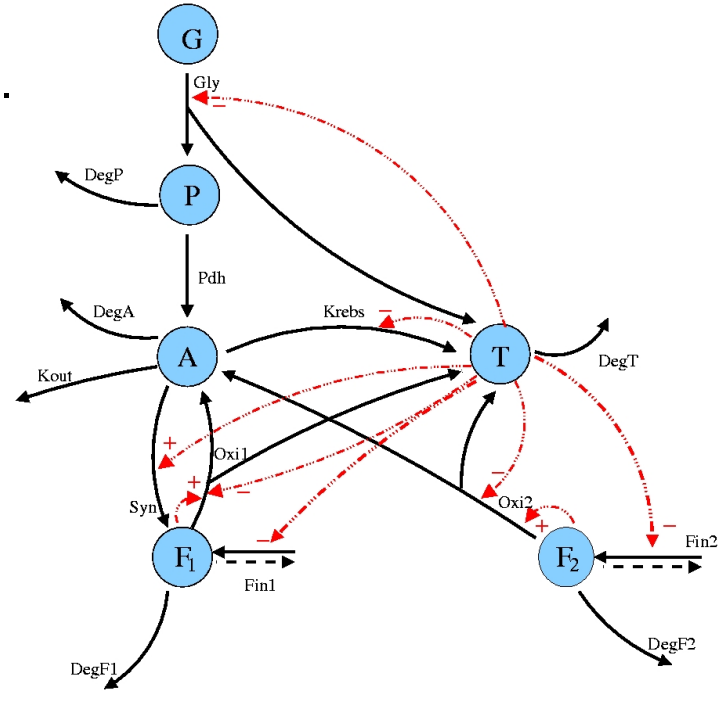
$\frac{\partial \text{ flux}}{\partial \text{ variable}}$	Gly	Pdh	Krebs	Kout	Syn	Oxi1	Oxi2	Fin1	Fin2	DegT	$\widetilde{\Psi}_1$	$\widetilde{\Psi}_2$	$\widetilde{\Psi}_3$	$\widetilde{\Psi}_4$	$\widetilde{\Psi}_5$	$\widetilde{\Psi}_6$
P	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	0	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0
F_1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

Constraints on the model : biological arguments

- Metabolic regulations
  - Substrate effect.
  - Passive or active transport effects.
- ATP constraints ; hormonal regulations
  - ATP increases ATP consumption [substrate effect]
  - Product negative feed-back : ATP controls negatively fluxes producing ATP [metabolic and hormonal response to glucagon]
  - control of ATP on de novo acids synthesis [substrate effect and insulin mediated stimulation]
  - Hormonal effect on fat intake : a drop in ATP stimulates lipolysis and fat intake [triggers glucagon and epinephrine production]
- Genetic regulations [long time scale]
  - Abstract enzymes E_i regulate their corresponding fluxes.
  - PUFA activates PPAR and inhibits active-LXR
  - LXR or PPAR triggers abstract enzymes production.
  - Degradation effects occurs on each genetic variable.

Metabolic (without genetic regulation) model

- Genetic regulation only occurs at long timescales.
- Non genetically regulated model (short timescales) : enzymes have a constant concentration.



$$
\begin{cases}
\frac{dP}{dt} = \mathrm{Gly}(G,T) - \delta_P P - \mathrm{Pdh}(P) \\
\frac{dA}{dt} = \mathrm{Pdh}(P) + \mathrm{Oxi1}_{qs}(F_1,F_2,T) + \mathrm{Oxi2}_{qs}(F_2,T) \\
\quad -\mathrm{Krebs}(A,T) - \mathrm{Kout}_{qs}(A,F_2) - \mathrm{Syn}_{qs}(A,F_2,T) - \delta_A A \\
\frac{dF_1}{dt} = \mathrm{Syn}_{qs}(A,F_2,T) - \mathrm{Oxi1}_{qs}(F_1,F_2,T) + \mathrm{Fin1}(F_1,T) - \delta_{F_1} F_1 \\
\frac{dF_2}{dt} = -\mathrm{Oxi2}_{qs}(F_2,T) + \mathrm{Fin2}(F_2,T) - \delta_{F_2} F_2 \\
\frac{dT}{dt} = \alpha_G \mathrm{Gly}(G,T) + \alpha_K \mathrm{Krebs}(A,T) + \alpha_{O1} \mathrm{Oxi1}_{qs}(F_1,F_2,T) \\
\quad +\alpha_{O2} \mathrm{Oxi2}_{qs}(F_2,T) - \mathrm{DegT}(T)
\end{cases}
$$

$\frac{\partial \text{ flux}}{\partial \text{ variable}}$	Gly	Pdh	Krebs	Kout_{qs}	Syn_{qs}	$\mathrm{Oxi1}_{qs}$	$\mathrm{Oxi2}_{qs}$	Fin1	Fin2	DegT
P	0	+	0	0	0	0	0	0	0	0
A	0	0	+	+	+	0	0	0	0	0
F_1	0	0	0	0	0	+	0	−	0	0
F_2	0	0	0	0	0	0	+	0	−	0
T	−	0	−	0	+	−	−	−	−	+
G	+	0	0	0	0	0	0	0	0	0

Generic model : illustrations

Quantitative versions are used as illustrations of robust dynamical behaviors.

- Rather generic choice of the form of the functions, including numerical constants.
- Check that the constraints are satisfied.
- Low complexity abstractions : only robust features of dynamics of the model are meaningful.

$$
\begin{cases}
\frac{dP}{dt} = \frac{l_{Gly}}{L_{Gly}+T^2}\frac{k_{Gly}G}{K_{Gly}+G} - \frac{k_{Pdh}P}{K_{Pdh}+P} - \delta_P P \\
\frac{dA}{dt} = \frac{k_{Pdh}P}{K_{Pdh}+P} + \frac{l_{Oxi1}}{L_{Oxi1}+T^2}\frac{k_{Oxi1}E_2F_1}{K_{Oxi1}+F_1} + \frac{l_{Oxi2}}{L_{Oxi2}+T^2}\frac{k_{Oxi2}E_3F_2}{K_{Oxi2}+F_2} - \frac{l_{Krebs}}{L_{Krebs}+T^2}\frac{k_{Krebs}A}{K_{Krebs}+A} \\
\quad - \frac{k_{Kout}E_4A}{K_{Kout}+A} - \frac{k_{Syn}E_1A}{K_{Syn}+A}\frac{l_{Syn}T^2}{L_{Syn}+T^2} - \delta_A A \\
\frac{dF_1}{dt} = \frac{k_{Syn}E_1A}{K_{Syn}+A}\frac{l_{Syn}T^2}{L_{Syn}+T^2} - k_{Fin1}F_1 + \frac{l_{Fin1}}{L_{Fin1}+T^2} - \frac{l_{Oxi1}}{L_{Oxi1}+T^2}\frac{k_{Oxi1}E_2F_1}{K_{Oxi1}+F_1} - \delta_{F_1}F_1 \\
\frac{dF_2}{dt} = -k_{Fin2}F_2 + \frac{l_{Fin2}}{L_{Fin2}+T^2} - \frac{l_{Oxi2}}{L_{Oxi2}+T^2}\frac{k_{Oxi2}E_3F_2}{K_{Oxi2}+F_2} - \delta_{F_2}F_2 \\
\frac{dT}{dt} = \alpha_G\frac{l_{Gly}}{L_{Gly}+T^2}\frac{k_{Gly}G}{K_{Gly}+G} + \alpha_K\frac{l_{Krebs}}{L_{Krebs}+T^2}\frac{k_{Krebs}A}{K_{Krebs}+A} + \alpha_{O1}\frac{l_{Oxi1}}{L_{Oxi1}+T^2}\frac{k_{Oxi1}E_2F_1}{K_{Oxi1}+F_1} \\
\quad + \alpha_{O2}\frac{l_{Oxi2}}{L_{Oxi2}+T^2}\frac{k_{Oxi2}E_3F_2}{K_{Oxi2}+F_2} - \delta_T T \\
\frac{dPP}{dt} = \frac{k_{PP}F_2^{a_{PP}}}{K_{PP}+F_2^{a_{PP}}} \quad \cdots + b_{PP}
\end{cases}
$$

Predictions of the model

Existence of an equilibrium

Proposition 1 : *The genetically regulated model of lipid metabolism admits at least a quasi-stationnary state and an equilibrium state for every parameter G, provided that the following conditions are satisfied*

- Fluxes are irreversible [except fatty acids intake/outake]
- The fluxes satisfy the differential constraints
- Degradation terms are linear (except T)
- Irreversible fluxes vanish when there is no substrate.
- All fluxes except degradation saturate at high concentrations
- ATP consumption is an increasing function of ATP with no saturation effect [cells can not store ATP]
- Recovery effect on each metabolic variable : if a variable is zero, then at leastone elementary flux that produces the variable is activated [if the cell contains no PUFA, then PUFA enter the cell]

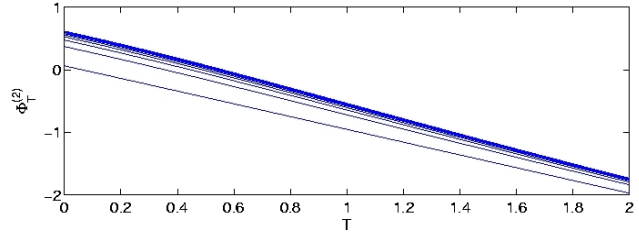
Uniqueness of equilibrium

- The interaction graph of the model always has a positive circuit.
- Compute a sufficient condition for unicity of equilibrium in terms of derivatives of fluxes.

Proposition 2 : *Both the equilibrium and the quasi-stationnary state of the model are unique if the following condition is fulfilled*

Mathematic condition $\frac{d\Phi_T^{(2)}}{dT} < 0$

$$\frac{d\Phi_T^{(2)}}{dT} = \alpha_{\mathrm{G}} \frac{\partial \mathrm{Gly}}{\partial \mathrm{T}} + \alpha_{\mathrm{K}} \left(\frac{\partial \mathrm{Krebs}}{\partial \mathrm{A}} \frac{\partial \mathrm{A}^{(2)}}{\partial \mathrm{T}} + \frac{\partial \mathrm{Krebs}}{\partial \mathrm{T}} \right) + \alpha_{\mathrm{O1}} \left(\frac{\partial \mathrm{Oxi1}}{\partial \mathrm{F_1}} \frac{\partial \mathrm{F_1}^{(2)}}{\partial \mathrm{T}} + \frac{\partial \mathrm{Oxi1}}{\partial \mathrm{F_2}} \frac{\partial \mathrm{F_2}^{(2)}}{\partial \mathrm{T}} + \frac{\partial \mathrm{Oxi1}}{\partial \mathrm{T}} \right) + \alpha_{\mathrm{O2}} \left(\frac{\partial \mathrm{Oxi2}}{\partial \mathrm{F_2}} \frac{\partial \mathrm{F_2}^{(2)}}{\partial \mathrm{T}} + \frac{\partial \mathrm{Oxi2}}{\partial \mathrm{T}} \right) - \delta_{\mathrm{T}}$$

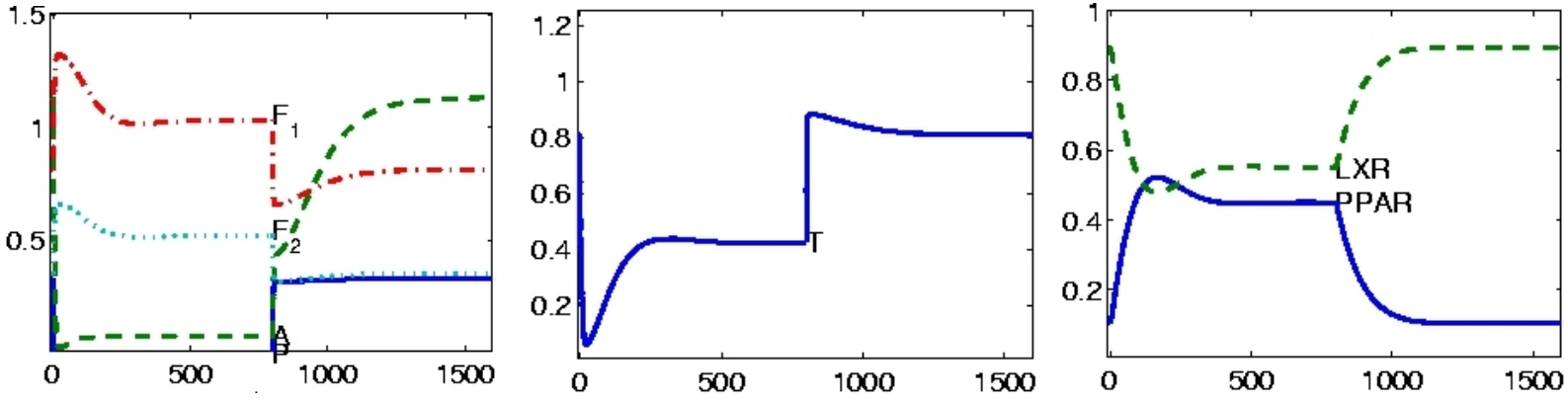


Numerical checking

Biological sufficient conditions

- Condition on ATP production : oxidation produces much more energy than the Krebs cycle
- Strong lipolytic response condition : when energy is forced to decrease, the total amount of fatty acids increases [intake (insured by lipolysis) overcomes outtake and consumption]

Simulation : fasting/refeeding protocols



Fasting up to t=750 ; followed by refeeding

- increase of fatty acids (equilibrium value) after fasting
- overshoot after the beginning of fasting ; undershoot after the beginning of refeeding
- Energy (ATP) has an abrupt fall, then it recovers slowly as a result of oxidation
- LXR (equilibrium value) diminishes and PPAR is amplified at fasting

Prediction : fatty acids concentration increase at fasting

The following properties are valid for rapid (at quasi-stationarity) as well as for slow (at equilibrium) response as soon as unicity and extra conditions are fulfilled

Prediction 1 : *ATP decreases during fasting and increases during feeding.*

Biological observation *mass of regulating PUFA in the hepatic cell increase during fasting (Lee et al., 2004)*

Prediction 2 : *PUFA increase at fasting iff the intake control overcomes the oxidation control for PUFA.*

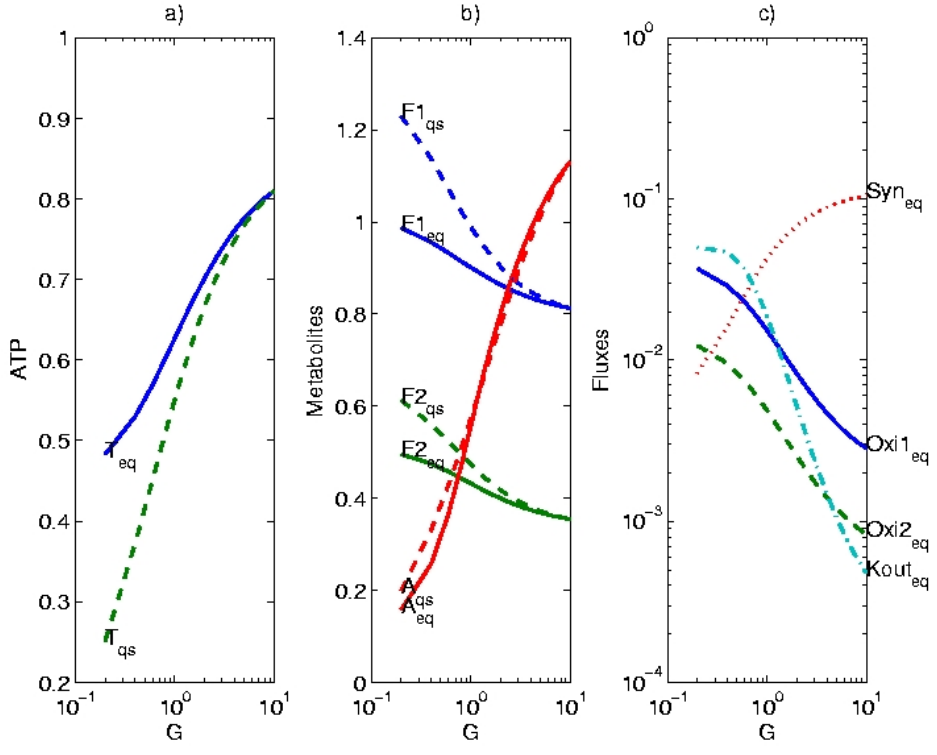
Prediction 3 : *The curves representing PUFA concentration during refeeding must show an overshoot* : the increase in concentration is greater immediately at quasi-stationarity than later at equilibrium.

$$\left(\left|\frac{d\mathrm{F}_2{}^{(3)}}{d\mathrm{G}}\right|_{qs} > \left|\frac{d\mathrm{F}_2{}^{(3)}}{d\mathrm{G}}\right|_{eq}\right)$$

Simulation : response curves when food G is changing

- Energy T increases with food, fatty acids concentrations decrease with food
- antagonistic relation between synthesis and oxidation : when food G decreases, the synthesis dominated regime changes to an oxidation dominated regime
- buffering effect (effet tampon) : energy T is not zero when food G is zero
- Strong buffering effect : the slope of the dependence of T on G is weaker at equilibrium than at quasi-stationarity.

Genetic regulation increases buffering.



Prediction : genetic regulation reinforces energy buffering at fasting

If unicity conditions and control conditions are fulfilled :

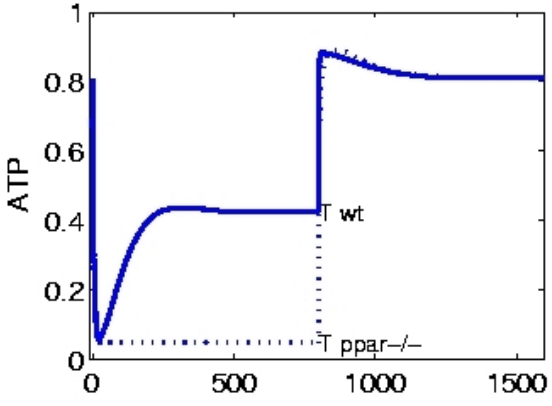
- Strong lipolytic response condition and condition on the ATP production,
- $\left(R_{\mathrm{T}}^{\mathrm{Fin2}} - R_{\mathrm{T}}^{\mathrm{Oxi2}}\right)_{eq,qs} > 0$,
- $\left(\frac{1-\rho_{\mathrm{F}_1}^{\mathrm{Oxi1}}}{\rho_{\mathrm{F}_1}^{\mathrm{Oxi1}}} R_{\mathrm{F}_2}^{\mathrm{Oxi1}} - (1-\rho_{\mathrm{A}}^{\mathrm{Syn}}) R_{\mathrm{F}_2}^{\mathrm{Syn}} - \rho_{\mathrm{A}}^{\mathrm{Syn}} R_{\mathrm{F}_2}^{\mathrm{Kout}}\right)_{eq} > 0$

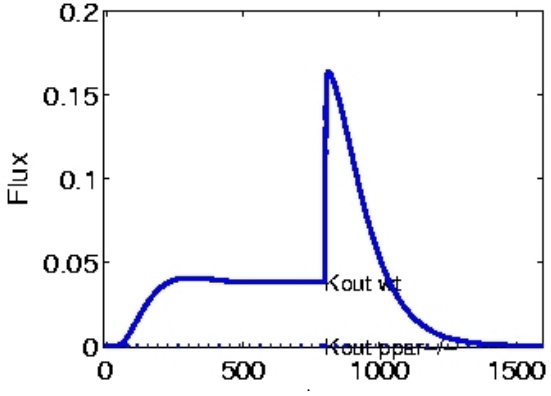
Prediction 4. *Then genetic regulation reinforces the energy buffering effect*

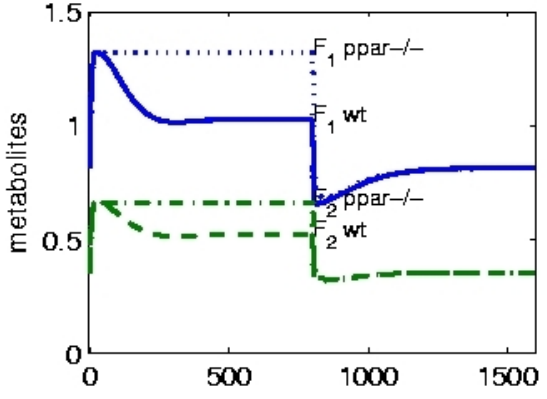
- The buffering effect is the variation of T for a fixed variation of G.
- Increasing energy variations is performed by boosting oxidation at fasting.
- Stimulating the decrease of T with the decrease of G is performed by the energy losses by ketone exits and diminished synthesis.
- $\left(\frac{d\mathrm{T}^{(3)}}{d\mathrm{G}}\right)_{qs} > \left(\frac{d\mathrm{T}^{(3)}}{d\mathrm{G}}\right)_{eq}$

Simulation : Fasting/refeeding protocol for a PPAR-/- mutant compared to wild-type

PPAR knock-out : no longer genetic control on oxidation.







the enzymes E_2, E_3, E_4 controlled by PPAR have constant, unadjustable values

dot curves : mutant type

- incapacity to recover energy on fasting : inefficient oxidation
- no ketone production : E_4 is not produced in mutants
- the fatty acids increase is accentuated under fasting in mutants
- the overshoot is replaced by a flat plateau connecting quasi-stationary and equilibrium values

Predictions about PPAR knock-out

Biological Observation : Experiments on transgenic mice : 72h-fast, *fatty acids concentration increases at a higher extent in PPAR knocked-out cells* with respect to wild type cells [Barnouin 2004, Lee 2004]

Prediction 5 : *PUFA concentration increase under fasting is stronger in PPAR knocked-out cells compared to the same increase in wild type cells.*

$$\left|\frac{d\mathrm{F}_2{}^{(3)}}{d\mathrm{G}}\right|_{eq,PPAR-/-} > \left|\frac{d\mathrm{F}_2{}^{(3)}}{d\mathrm{G}}\right|_{eq,WT}.$$

Prediction 6 : *PPAR knock-out reduces energy buffering.*

$$\left(\frac{d\mathrm{T}^{(3)}}{d\mathrm{G}}\right)_{eq,PPAR-/-} > \left(\frac{d\mathrm{T}^{(3)}}{d\mathrm{G}}\right)_{eq,WT}$$

Mathematical framework : successive elimination of variables

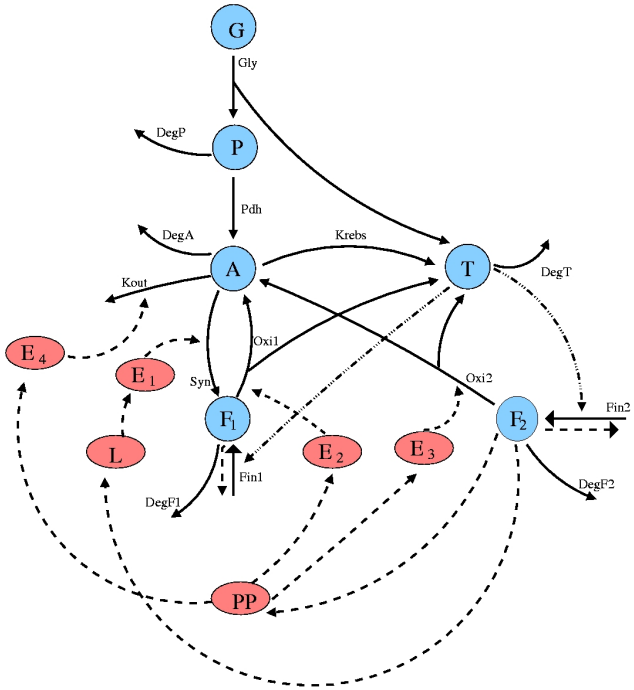
Study of equilibria

- (Main) Remark 1 : The predictions concern equilibria states and not dynamical properties.
- Remark 2 : Equilibria are characterized by a set of equations equal to zero.
- Remark 3 : One can equilibriate any equation before any other. This does not change the equilibrium state at the end of the process.

Successive reduction of models !

- Successively eliminate one or several variables.
- Criterion of elimination : existence and unicity of the variable at equilibrium with respect to the remaining variables.
- Compute the constraints of the new model by using the implicit fonction theorem.
- The reduced models have no dynamical meaning but they have the same equilibrium state as the first model.
- The order of reduction can be not intuitive : first reduce the genetic variables. Then some metabolites...

equilibration of genetic variables



$$\begin{cases} \frac{dP}{dt} = \mathrm{Gly}(G,T) - \dots \\ \frac{dA}{dt} = \mathrm{Pdh}(P) + \dots \\ \frac{dF_1}{dt} = \mathrm{Syn}(A,T,E_1) - \dots \\ \frac{dF_2}{dt} = -\mathrm{Oxi2}(F_2,T,E_3) + \dots \\ \frac{dT}{dt} = \alpha_G \mathrm{Gly}(G,T) + \dots \\ \frac{dPP}{dt} = \widetilde{\Psi}_1(F_2) - \delta_{PP} PP \\ \frac{dL}{dt} = \widetilde{\Psi}_2(F_2) - \delta_L L \\ \frac{dE_1}{dt} = \widetilde{\Psi}_3(L) - \delta_{E_1} E_1 \\ \frac{dE_2}{dt} = \widetilde{\Psi}_4(PP) - \delta_{E_2} E_2 \\ \frac{dE_3}{dt} = \widetilde{\Psi}_5(PP) - \delta_{E_3} E_3 \\ \frac{dE_4}{dt} = \widetilde{\Psi}_6(PP) - \delta_{E_4} E_4 \end{cases}$$

equilibriate genetic variables

$$\begin{cases} \frac{dPP}{dt} = \widetilde{\Psi}_1(F_2) - \delta_{PP} PP & = 0 \\ \frac{dL}{dt} = \widetilde{\Psi}_2(F_2) - \delta_L L & = 0 \\ \frac{dE_1}{dt} = \widetilde{\Psi}_3(L) - \delta_{E_1} E_1 & = 0 \\ \frac{dE_2}{dt} = \widetilde{\Psi}_4(PP) - \delta_{E_2} E_2 & = 0 \\ \frac{dE_3}{dt} = \widetilde{\Psi}_5(PP) - \delta_{E_3} E_3 & = 0 \\ \frac{dE_4}{dt} = \widetilde{\Psi}_6(PP) - \delta_{E_4} E_4 & = 0 \end{cases}$$

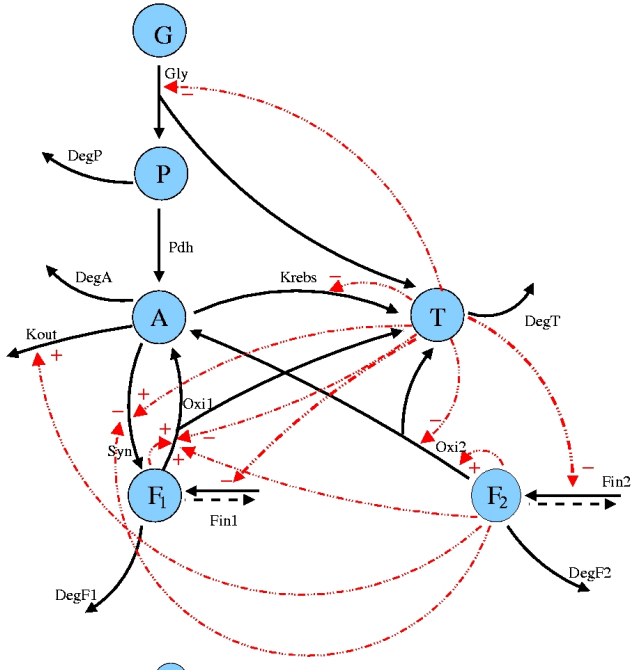
existence and unicity of a solution with respect to the other variables

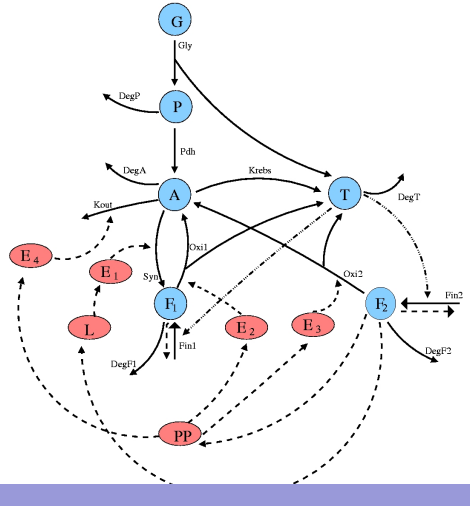
$$PP_{peq}, L_{peq}, E_{1peq}, E_{2peq}, E_{3peq}, E_{4peq} = \text{fonction}(F_2)$$

$$\frac{\partial Syn_{peq}}{\partial F_2} < 0, \qquad \frac{\partial Oxi1_{peq}}{\partial F_2} > 0$$

$$\frac{\partial Oxi2_{peq}}{\partial F_2} > 0, \qquad \frac{\partial Kout_{peq}}{\partial F_2} > 0.$$

Partial equilibrium model





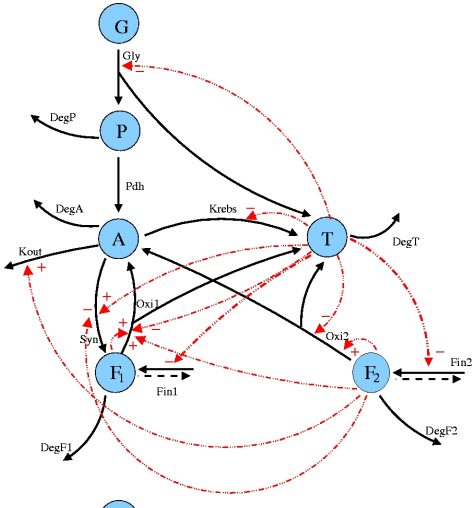
- Model of lipid metabolism in which genetic variables are equilibrated
- The constraint table integrate the implicit equilibrated constraints.
- The model has no dynamical meaning
- Same equilibrium states than the extended model

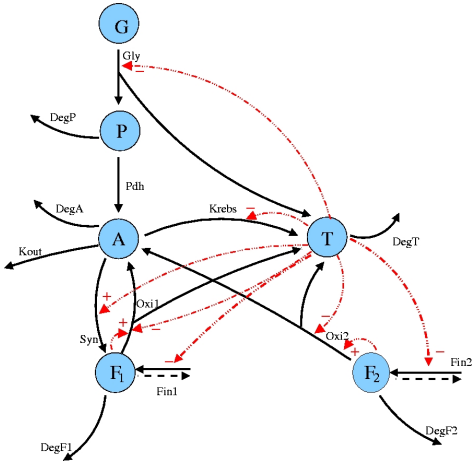
$$
\begin{cases}
\frac{dP}{dt} &= \mathrm{Gly}(G,T) - \delta_P P - \mathrm{Pdh}(P) \\
\frac{dA}{dt} &= \mathrm{Pdh}(P) + \mathrm{Oxi1}_{\mathrm{peq}}(F_1,F_2,T) + \mathrm{Oxi2}_{\mathrm{peq}}(F_2,T) \\
&\quad -\mathrm{Krebs}(A,T) - \mathrm{Kout}_{\mathrm{peq}}(A,F_2) - \mathrm{Syn}_{\mathrm{peq}}(A,F_2,T) - \delta \\
\frac{dF_1}{dt} &= \mathrm{Syn}_{\mathrm{peq}}(A,F_2,T) - \mathrm{Oxi1}_{\mathrm{peq}}(F_1,F_2,T) + \mathrm{Fin1}(F_1,T) - \delta \\
\frac{dF_2}{dt} &= -\mathrm{Oxi2}_{\mathrm{peq}}(F_2,T) + \mathrm{Fin2}(F_2,T) - \delta_{F_2} F_2 \\
\frac{dT}{dt} &= \alpha_G \mathrm{Gly}(G,T) + \alpha_K \mathrm{Krebs}(A,T) + \alpha_{O1} \mathrm{Oxi1}_{\mathrm{peq}}(F_1,F_2,T \\
&\quad +\alpha_{O2} \mathrm{Oxi2}_{\mathrm{peq}}(F_2,T) - \mathrm{DegT}(T)
\end{cases}
$$

$\frac{\partial \text{ flux}}{\partial \text{ variable}}$	Gly	Pdh	Krebs	$\mathrm{Kout}_{\mathrm{peq}}$	$\mathrm{Syn}_{\mathrm{peq}}$	$\mathrm{Oxi1}_{\mathrm{peq}}$	$\mathrm{Oxi2}_{\mathrm{peq}}$	Fin1	Fin2	De
P	0	+	0	0	0	0	0	0	0	0
A	0	0	+	+	+	0	0	0	0	0
F_1	0	0	0	0	0	+	0	−	0	0
F_2	0	0	0	+	−	+	+	0	−	0
T	−	0	−	0	+	−	−	−	−	+
G	+	0	0	0	0	0	0	0	0	0

Compare non genetically and genetically regulated models

The equilibrium states of the non genetically regulated and partial equilibriate models check the qame equations with a different table of constraints.





$$
\begin{cases}
\frac{dP}{dt} &= \mathrm{Gly(G,T)} - \delta_P P - \mathrm{Pdh(P)} \\
\frac{dA}{dt} &= \mathrm{Pdh(P)} + \mathrm{Oxi1_{peq,}}_{gnr}\mathrm{(F_1,F_2,T)} + \mathrm{Oxi2_{peq,}}_{gnr}\mathrm{(F_2,T)} \\
&\quad -\mathrm{Krebs(A,T)} - \mathrm{Kout_{peq,}}_{gnr}\mathrm{(A,F_2)} - \mathrm{Syn_{peq,}}_{gnr}\mathrm{(A,F_2,T)} - \delta \\
\frac{dF_1}{dt} &= \mathrm{Syn_{peq,}}_{gnr}\mathrm{(A,F_2,T)} - \mathrm{Oxi1_{peq,}}_{gnr}\mathrm{(F_1,F_2,T)} + \mathrm{Fin1}(F_1,T) - \\
\frac{dF_2}{dt} &= -\mathrm{Oxi2_{peq,}}_{gnr}\mathrm{(F_2,T)} + \mathrm{Fin2}(F_2,T) - \delta_{F_2} F_2 \\
\frac{dT}{dt} &= \alpha_G \mathrm{Gly(G,T)} + \alpha_K \mathrm{Krebs(A,T)} + \alpha_{O1} \mathrm{Oxi1_{peq,}}_{gnr}\mathrm{(F_1,F_2,T)} \\
&\quad +\alpha_{O2} \mathrm{Oxi2_{peq,}}_{gnr}\mathrm{(F_2,T)} - \mathrm{DegT(T)}
\end{cases}
$$

$\frac{\partial \text{ flux}}{\partial \text{ variable}}$	Gly	Pdh	Krebs	$\mathrm{Kout_{peq}}$ *gnr*	$\mathrm{Syn_{peq}}$ *gnr*	$\mathrm{Oxi1_{peq}}$ *gnr*	$\mathrm{Oxi2_{peq}}$ *gnr*	Fin1	Fin2	DegT
P	0	+	0	0	0	0	0	0	0	0
A	0	0	+	+	+	0	0	0	0	0
F_1	0	0	0	0	0	+	0	−	0	0
F_2 gnr	0	0	0	0	0	0	+	0	−	0
F_2 peq				+	−	+				
T	−	0	−	0	+	−	−	−	−	+
G	+	0	0	0	0	0	0	0	0	0

Compare the equilibrium states of the models to understand the role of genetic regulations

Elimination of metabolic variables

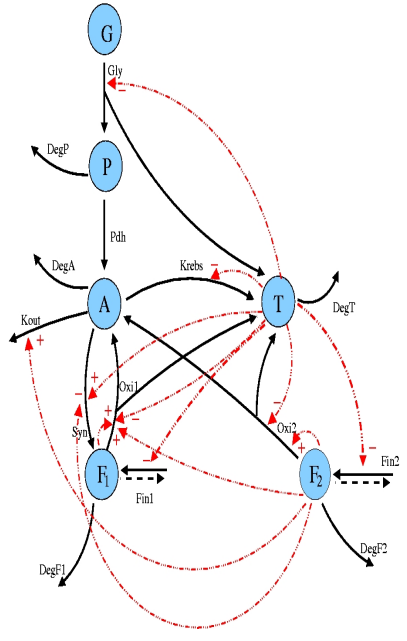
- Eliminate P . The equation $\Phi_{\mathrm{P}}(\mathrm{G},\mathrm{P},\mathrm{T})=0$ has a unique solution $\mathrm{P}^{(1)}(\mathrm{G},\mathrm{T})$ for every fixed (G,T) (unicity deduced from $\frac{\partial\Phi_{\mathrm{P}}}{\partial\mathrm{P}}<0$)
- Eliminate A, F_1, F_2

  $\Phi_{\mathrm{A}}(\mathrm{P}^{(1)}(\mathrm{G},\mathrm{T}),\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T})=0;\ \Phi_{\mathrm{F}_1}(\mathrm{A},\mathrm{F}_1,\mathrm{F}_2,\mathrm{T})=0;\ \Phi_{\mathrm{F}_2}(\mathrm{F}_2,\mathrm{T})=0$

  The system has a unique solution $(\mathrm{A}^{(2)}(\mathrm{G},\mathrm{T}),\mathrm{F}_1{}^{(2)}(\mathrm{G},\mathrm{T}),\mathrm{F}_2{}^{(2)}(\mathrm{G},\mathrm{T}))$ because of the Gale-Nikaido theorem.
- Gale-Nikaido : If $(x,y,z)\to(f_x,f_y,f_z)$ is differentiable onto $\mathbb{R}^3_+$, of Jacobian J, such that all the principal minors of $-J$ are positive, the system $f_x=f_y=f_z=0$ has a unique solution if a solution exists.
- Reduce T with respect to G

  $\Phi_{\mathrm{T}}(\mathrm{G},\mathrm{A}^{(2)}(\mathrm{G},\mathrm{T}),\mathrm{F}_1{}^{(2)}(\mathrm{G},\mathrm{T}),\mathrm{F}_2{}^{(2)}(\mathrm{G},\mathrm{T}),\mathrm{T})=0$

  Unicity condition : sufficient condition for $\frac{d\Phi_{\mathrm{T}}}{d\mathrm{T}}<0$.
- If the unicity condition is satisfied, compare the signs of the equilibrated values in different models (non genetically regulated, PPAR mutant...).



Conclusion

- Abstract model : only 12 variables to describe the regulations and main fluxes in lipid metabolism.
- Qualitative model : differential equations together with a table of constraints on elementary fluxes.
- Simulations : provide rough behavior of the system
- Reduction method : allows to make predictions on the static properties of model even if the model is not explicit.
- Compatible with observations : behavior of fatty acids ; PPAR mutants ; understand the role of regulation.

To do

- Find the most efficient sequence of equilibration
- Study the stability of equilibria
- Distinguish between the different types of fatty acids
- Relation with extended models (KEGG and genetic regulations)
- Other species or tissues ?