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Kinetic Model of Human Hepatocyte Glycolysis, Gluconeogenesis & Glycogen metabolism

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Abstract

A kinetic model of the human core hepatocyte metabolism is formulated consisting of energy and carbohydrate metabolism, amino acid synthesis and degradation, nucleotide and fatty acid metabolism. This kinetic network enables us to study characteristic hepatocyte functionality like blood glucose homoeostasis or NH_3 detoxification.

Kinetic Model

The core metabolism of the human hepatocyte was reconstructed in a compartmentalized model and validated with network- and flux-based methods like FBA and dead end analysis. The kinetic modelling has been started for a subsystem consisting of glycolysis, gluconeogenesis and glycogen metabolism [Fig.1]. The basic kinetic model can be successively extended to the complete central metabolism.

Switching between metabolic states

One of the mayor functions of the liver is blood glucose homoeostasis. Hepatocytes can increase or decrease the blood glucose level depending on the blood metabolite concentrations and hormonal signals like glucagon and insulin. Here an analysis of switching between different states (glycolysis/ gluconeogenesis) is presented based on phosphorylation/ dephosphorylation of key enzymes.

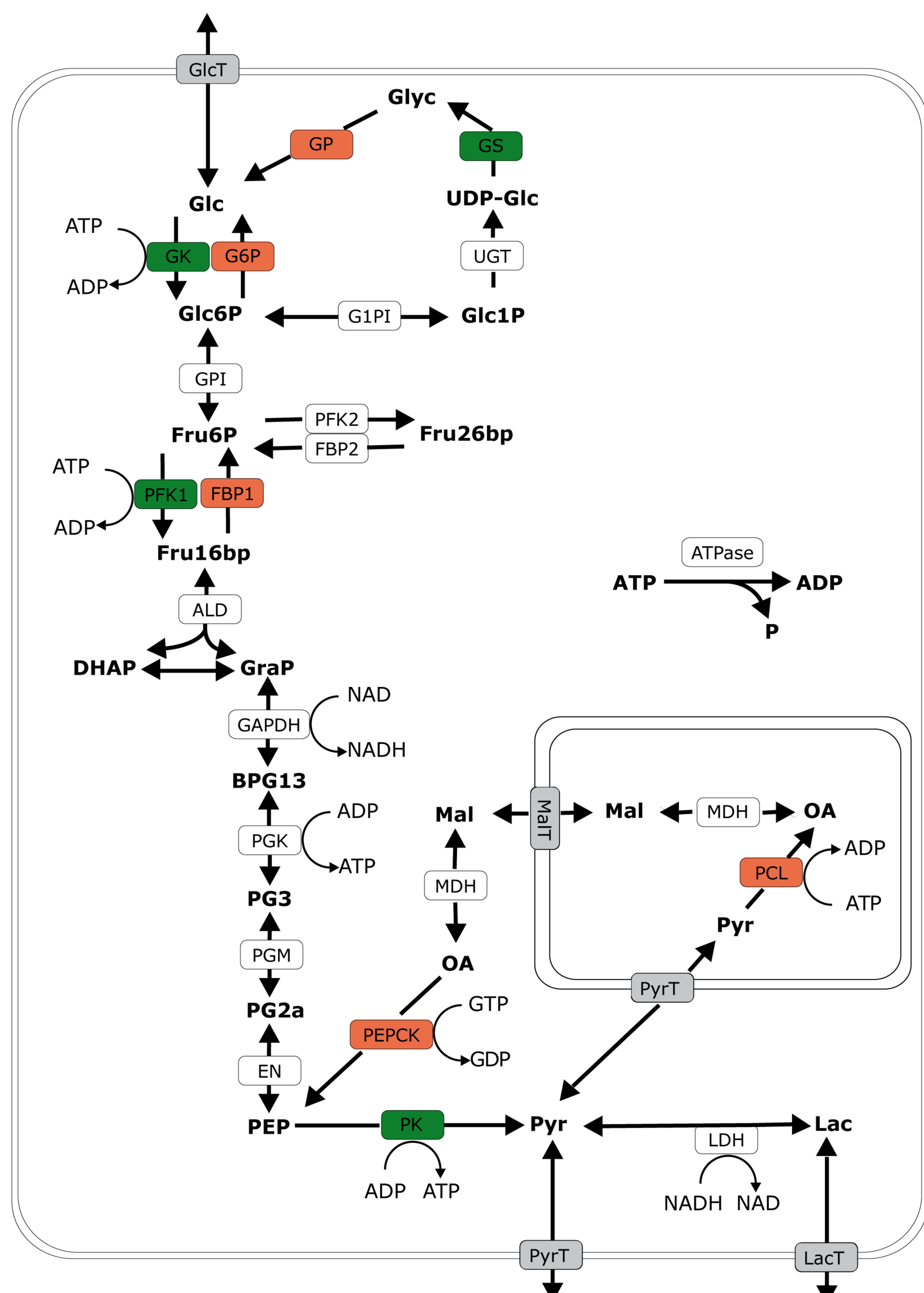


Fig.1: Overview of kinetic model glycolysis, gluconeogenesis and glycogen metabolism. The counteracting enzymes for glucose consumption/storage and glucose production are marked in green and red respectively. The model is compartmentalized in cytosol, blood stream and mitochondrium. Some of the reactions for the gluconeogenesis are localized in the mitochondrium.

PFK1 & FBP1 switch

Hepatocytes contain rate-controlling enzymes specific for gluconeogenesis (G6P, FBP1, PEPCK) and glycolysis (GK, PFK1, PK) which makes a sensitive control system possible. These enzyme pairs are regulated in counteracting ways to switch between glycolysis and gluconeogenesis [3]. The regulation mechanism [Fig.2] of the PFK1/FBP1 switch is based on different kinetic properties of the bifunctional PFK2/FBP2 enzyme (BFE) in the phosphorylated and unphosphorylated form [Fig.3]. The fraction of phosphorylated BFE γ = (phosphorylated BFE / total BFE) regulates the amount of the important PFK1 activator and FBP1 inhibitor fructose-2,6-bisphosphate (fru26bp).

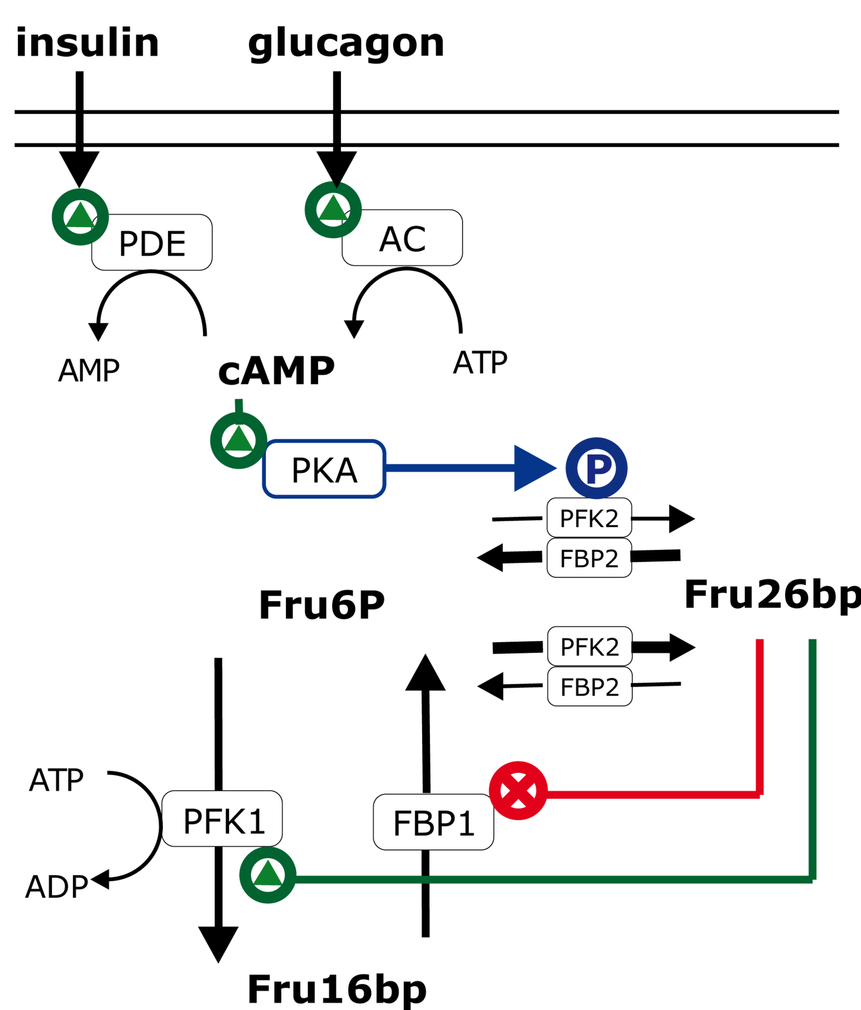


Fig.2: Regulation of the PFK1/FBP1 switch Hormonal signals like insulin and glucagon are converted into internal cAMP signals via phosphodiesterase (PDE) and adenylate cyclase (AC). The cAMP level changes the phosphorylation state of the bifunctional enzyme (BFE) via cAMP dependent proteinkinase (PKA) which is activated by cAMP.
The phosphorylated and unphosphorylated ('native') BFE have different kinetic properties for the FBP2 and PFK2 subunit, respectively [Fig.3, 4]. In the phosphorylated state the synthesis of fru26bp is increased and the degradation decreased. Fru26bp is an important activator of PFK1 (glycolysis) and inactivator of FBPase1 (gluconeogenesis). By changing the fraction of phosphorylated BFE (γ) the fru26bp is changed and the direction of flux through glycolysis / gluconeogenesis modified accordingly.

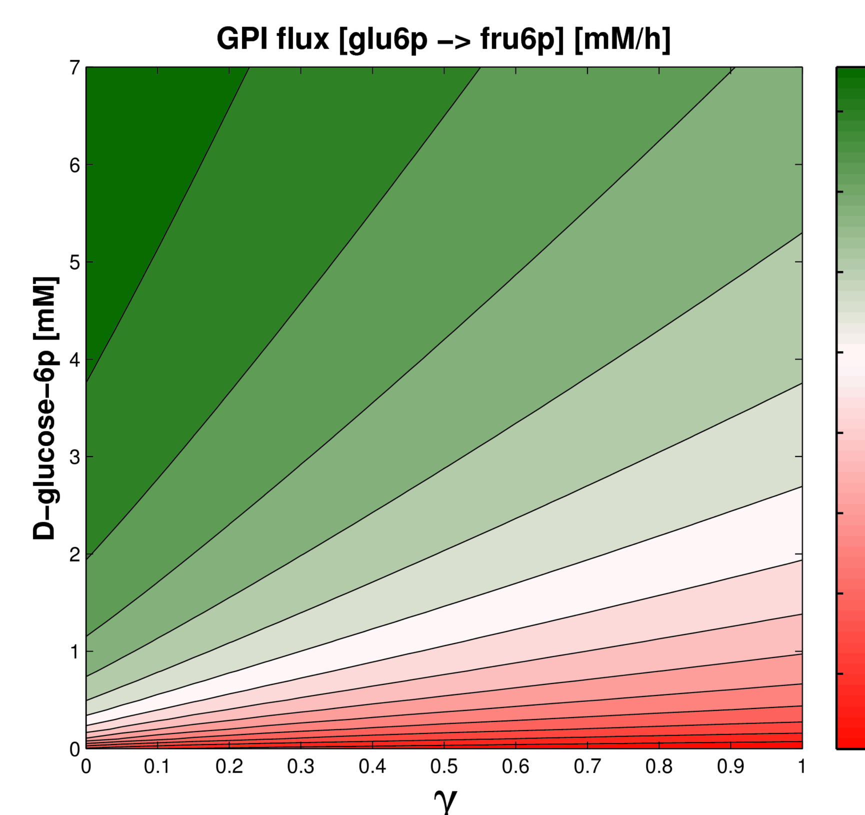
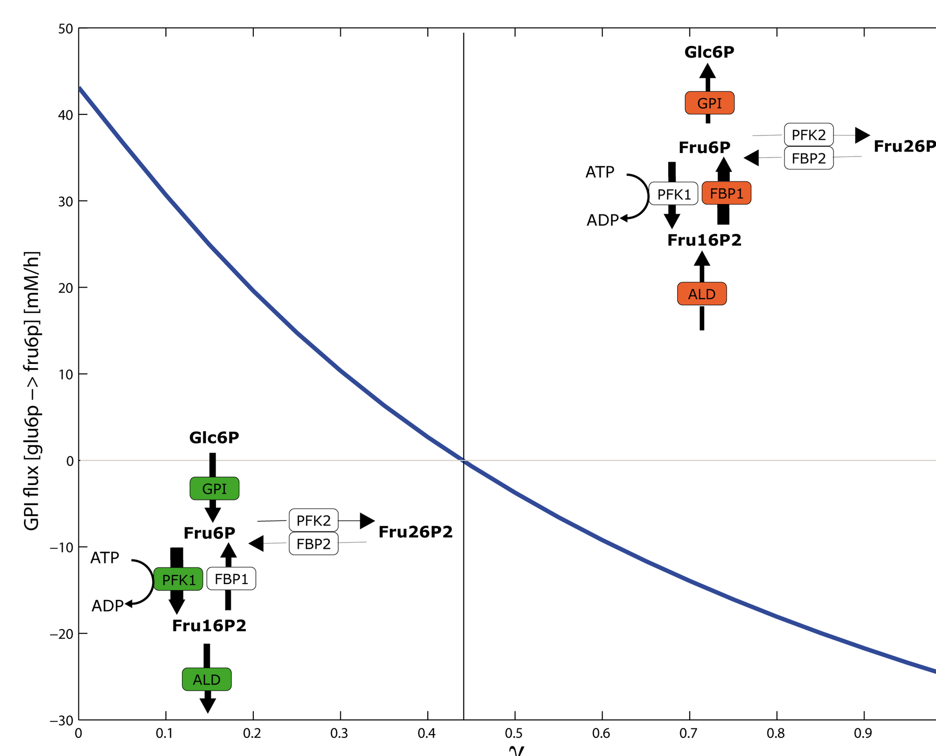


Fig.4: Switching between glycolysis and gluconeogenesis depending on the phosphorylation state of the BFE: Flux through the glucose-6-p isomerase (GPI) reaction, which is identical to the flux through glycolysis or gluconeogenesis respectively [Fig.1]. In green positive fluxes through the GPI (glycolysis), in red negative fluxes (gluconeogenesis). Switching can be achieved by changing the phosphorylation state of the BFE γ (left to right). The switching is not only dependent on γ but also on the metabolic state (glu6p concentration). The switching point depends considerably on the glu6p: For high glu6p no switching from glycolysis to gluconeogenesis can be achieved, for low glu6p concentrations no switching from gluconeogenesis to glycolysis.



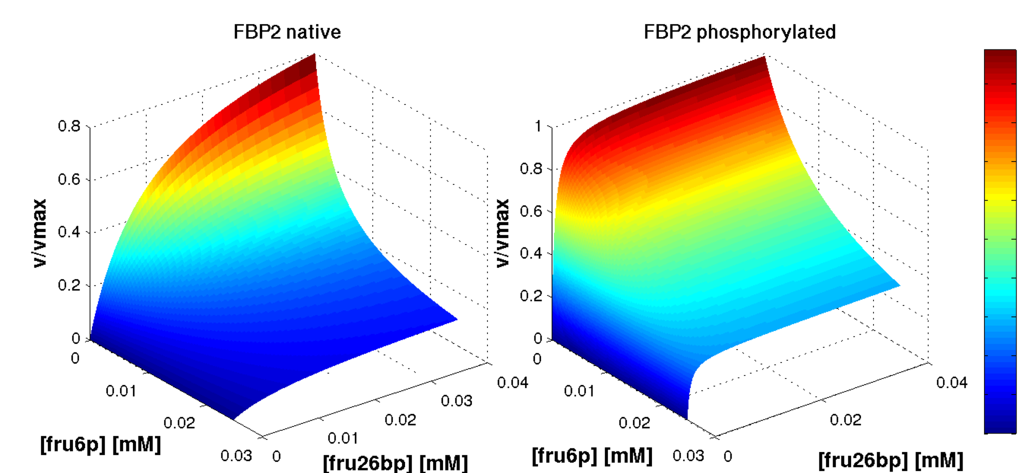


Fig.3: **Different kinetic properties of FBP2 for the native and the phosphorylated form** [4] . The different relative reaction rates for the FBP2 are displayed depending on the substrate fru26bp and the inhibitor fru6p. The kinetics of the native and phosphorylated form differ considerably and can so build a switch. Like the FBP2, the PFK2 also differs notably in its native and phosphorylated kinetic properties [4].

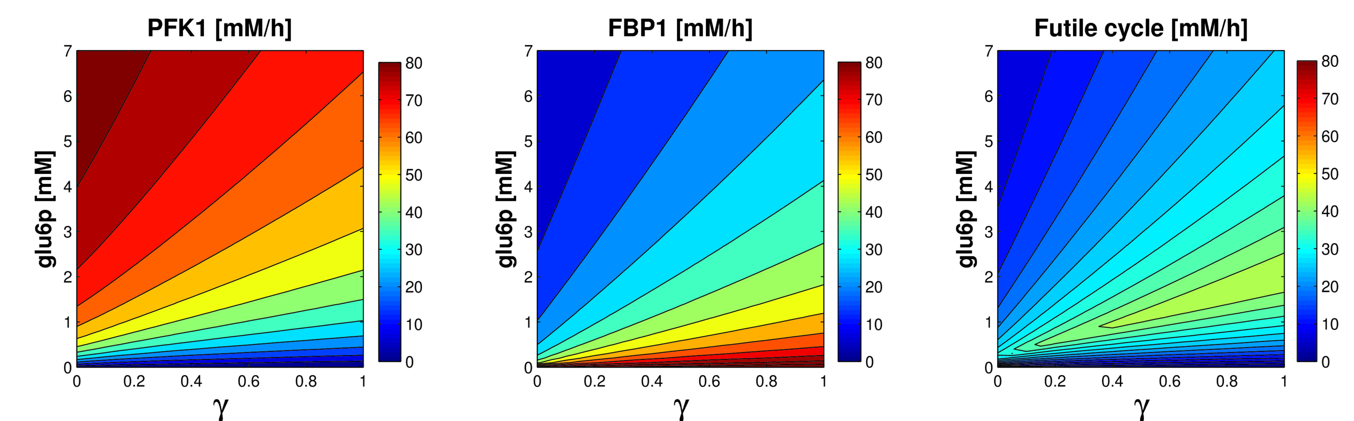


Fig.6: **Switching PFK1, FBP1 and futile cycle flux depending on phosphorylation state of BFE and the glucose-6p** In [Fig. 5] the switching can be seen for the PFK1 and the FBP1. PFK1 flux is high if the switch is in glycolysis position (γ~0) and the glu6p concentration is high and low if the switch is in gluconeogenesis (γ~1) position and the glu6p is low. The flux through the enzymes is reciprocal The futile cycle is maximal for glu6p concentrations in the range of 1mM and (γ~1). If switch and glu6p concentration favor the same direction of flux almost no energy waste via futile cycling occurs.

Switch analysis

By changing the fraction of phosphorylated key regulator enzyme (γ) the switching between different states can be achieved [Fig.4,5]. The switching depends not only on the amount of phosphorylated enzyme but also on the metabolic state of the system (metabolite concentrations).
If the glu6p level is high it is much more difficult to switch into gluconeogenesis than when the glu6p level is low. But the system can be easily switched into the glycolysis direction. On the other hand if the glu6p level is low it is much more difficult to switch into the glycolysis direction.

Futile cycle

The futile cycling and the according energy waste through the PFK1/FBP1 is minimal if the metabolite levels (glu6p) support the direction of the switch but are much higher if the metabolite levels counteract the switch direction [Fig.6].

Outlook

- Integration/expansion
  - Integration of other metabolic switches and long-time regulation via transcriptional regulation
- Study of hepatic response to dietary disturbances
- Analysis of metabolic disorders like diabetes

References:

[1] Kinetic hybrid models composed of mechanistic and simplified enzymatic rate laws, Sascha Bulik, Sergio Grimbs, Carola Huthmacher, Joachim Selbig and Hermann G. Holzhuetter; FEBS Journal (2008)
[2] Use of mathematical models for predicting the metabolic effect of large-scale enzyme activity alterations. Application to enzyme deficiencies of red blood cells.; Schuster R, Holzhutter HG; Eur J Biochem. 1995 Apr 15;229(2):403-18. (1995)
[3] Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis; S.J.Pilkis & D.K.Granner; Ann. Rev. Physiol. 1992, 54:885-909 (1992)
[4] Difference in kinetic properties in phospho and dephospho forms of FBPase2 and PFK2; Journal of biological chemistry, Vol.259, No 1 (1984)

[<] **Fig.5: Switching between glycolysis and gluconeogenesis for glUc6p = 1mM** The arrow thickness corresponds to the flux through the reactions for γ=0.1 (glycolysis) and γ=0.9 (gluconeogenesis). Under these conditions futile cycling can be observed, but the switch still works as expected.

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