Math. Model. Nat. Phenom.
Vol. X, No. X, 201x, pp. xxxx
DOI: xxxxxxxxxx

Computational modeling of quiescent platelet energy metabolism in the context of whole-body glucose turnover

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Abstract. Platelets are anucleate blood cells circulating in the bloodstream for up to 9 days in quiescent state. Upon vessel wall injury, platelets become activated, change their shape and adhere to the vessel wall and each other, thus forming a thrombus and preventing the blood loss. To get energy for these processes, they can use oxidative phosphorylation and glycolysis utilizing blood glucose, stored glycogen or fatty acids as fuel. Yet, there is no agreement in experimental data on platelet functioning in quiescent and activated states. This study is a systematic analysis of the energy abilities of quiescent platelets through mathematical modeling of their energy metabolism by Flux Balance Analysis (FBA). As a result of the FBA analysis we concluded that a platelet

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even in quiescent state utilizes blood glucose at high rate (0.1 mM/s), producing lactate from 99% of it and about 0.2 mM/s ATP from glycolysis and respiration. Such high fluxes of glucose are not always available due to platelet's glucose transporter (GLUT3) kinetic limitations. We positioned a "FBA" platelet in human glucose/insulin/glucagon PBPK/PD model to theoretically investigate platelet metabolism in close-to-real conditions. The main result of our study is that the stored glycogen could be daily used and resynthesized during platelet lifetime.

Keywords. blood platelets, blood glucose, glycogen, PB/PK, FBA
Mathematics Subject Classification: 92C05, 92C45, 92C42

1. Introduction

Platelets are small discoid megakaryocyte fragments circulating in the bloodstream for up to 9 days (at a concentration of 200-400 thousands of cells/μl). Their main function is to become activated and form hemostatic plugs in case of vessel wall injury. An activated platelet has active integrins, which give cells ability to adhere to vessel walls and each other, has long membrane pseudopodia (more surfaces to interact) and has the ability to induce other platelets activation by generating various soluble agonists. The activated platelets rapidly form an aggregate preventing blood loss [1]. Thus, platelet activation, proceeding in less than 5 sec, includes several energy-consuming processes. Although it is now generally believed that platelet energy metabolism is provided by both glycolysis (with lactate production) and oxidative phosphorylation, opinions differ on the relative importance of these two pathways in platelet energy metabolism in different states [2–4].

Even in their quiescent state, platelets seem to be unusually metabolically active, exhibiting high ATP turnover rates. The reported major energy substrates that contribute to ATP production thus far are glucose [2,5], glutamine [3,6] and fatty acids [3,7]. Reports on enzymatically measured platelet glucose consumption varied from 0.26 to 1.35 μmole/10^{11} cells/min, while lactate production rates varied from 0.36 to 4.22 umole/10^{11} cells/min [2,3,5,8–11]. Both rates depend on oxygen and other energy sources availability. This suggests that differences in the degree of oxygenation of the incubation medium may contribute to the wide variation in results due to metabolism regulation by oxygen

availability [2]. Interestingly, addition of albumin with fatty acids to the solution reduces rates of glucose consumption and lactate production by 30% on the average [2].

Another source of energy in platelet is glycogen. Experimental data prove that platelets contain more glycogen than skeletal muscle cell, from 43 to 65 μmole of glycosyl residues per 10^{11} cells [4,11–14]. Glycogen is a buffer stock of a glucose that is normally not consumed by cells in a quiescent state. Moreover, Akkerman et al. [13] suggests that platelets do not use glycogen until glucose concentrations are below 1 mM. The rate of measured glycogen consumption varies from 0.0158 to 0.385 μmol (of glycosil residues)/10^{11} cells/min [2,11,13].

Platelet respiration seems to be even less studied process. Rates of glucose oxidation were directly measured in several studies [3,5,7–9] and oxygen consumption due to glucose oxidation constitutes from 1 to 11% of the total oxidation. This indicates that some endogenous fuel may contribute to the oxygen consumption and energy production. Supplementation with the fatty acid palmitate increased respiration [2], whereas inhibition of fatty acids transport to mitochondria decreased respiration [3], indicating that platelets are able to transport extracellular fatty acids [15] and utilize them and endogenous fatty acids for respiration [16].

Thereby, despite the claimed quiescent platelet state, flux values vary widely and seem to be too big for the quiescent state in comparison with other cells [7]. To integrate the listed data, a Flux Balance Analysis of platelet energy metabolism could be performed for calculation of the distribution of energy fluxes among different ATP-producing pathways. The existing stoichiometric model of platelet metabolism constructed by Thomas et al. [17] provides a platform for such analysis although it does not include necessary information of the metabolic fluxes limitations. We used ATP production as objective function and analyzed several sets of quiescent platelet experimental data [2,3,7,9,13,18]. We found that even in quiescent state platelet functions close to its enzyme turnover limit, thus providing a question how platelet metabolism functions in human organism.

A simple PBPK (“Physiology based pharmacokinetics”) model of variations in human blood glucose concentration could provide a frame for assessment of platelet functioning in close-to-real conditions. Among variety of human glucose PBPK models the one of Markakis et al. [19] was chosen for its simplicity and good description of experimental data on blood glucose. The combined FBA and PBPK models provide a PK/PD

(pharmacokinetic/pharmacodynamics) model allowing to assess influence of daily meal (blood glucose) on human platelet metabolism.

2. Computational models

2.1. Flux Balance Analysis of platelet energetic metabolism

2.1.1. Key modeling assumptions.

To model metabolic fluxes, we used the FBA method, which intends to derive a set of steady-state metabolite fluxes through biochemical pathways that correspond to a particular "cellular objective", i.e. objective function [20]. The main assumption in FBA modeling is a quasi steady-state assumption, i.e. the metabolite concentrations and the reaction rates are assumed to be unchanged on the time scale under consideration [21]. Thus a system of ordinary differential equations usually describing a set of biochemical reactions is converted into a set of linear equations with reaction rates (fluxes) as variables. The stoichiometric coefficients of the reactions are used to form mass balance constraints around the metabolites, leading to the system of linear equations ($\mathbf{S}\times\mathbf{v}=0$, where $\mathbf{S}$ is stoichiometric matrix, $\mathbf{v}$ is the vector of variables (fluxes)). Other constraints are introduced by setting the lower and upper bounds of the associated fluxes emerging from reaction reversibility state, enzymes activities and measured metabolite rates [21]. Since the number of unknown reaction rates (metabolic fluxes) is usually higher than the number of metabolites, the system of linear equations is underdetermined, and thus it does not have a unique solution [22]. To obtain a unique flux distribution, an additional constraint should be added. In FBA, the investigator choses a vector of parameters, $\mathbf{f}$, called an objective function, and states that the solution should have the maximum value of $\mathbf{f}\times\mathbf{v}$. The linear programming techniques are used to find the optimal solution with respect to the chosen objective function [23]. Commonly chosen objective functions for bacterial FBA models are biomass production [20,23] as assumed bacterium's life objective is to grow. Here we have chosen the ATP production flux as the objective function because it allows investigation of the limitations of platelet energy metabolism.

2.1.2. Metabolic pathways and parameters used in model construction

The metabolic space of platelet energetic metabolism contained 88 reconstructed reactions and 87 compounds. In short, model included reactions and reactants of the following

metabolic pathways: glycolysis, glycogenolysis, penthose phosphate pathway and tricarboxylic acid cycle (62 reaction fully taken out of 1008 from Thomas et al. study [17]) with addition of fatty acid transport and β-oxidation, oxidative phosphorylation and oxidative stress reconstructed using KEGG database for Homo Sapience [22] and platelet proteomics data [24]. In the latter part all sequential reactions were replaced with a single merged reaction. For model details and scheme of major reactions, see Supplemental Files 1 and 2, respectively. Boundaries for the rates of most reactions were are unknown, we only considered reaction directions as model constraints.

Metabolic flux distributions were calculated by using measured glucose, fatty acid and oxygen uptake, glycogen utilization and lactate production rates as constraints in the model. All fluxes were recalculated from μmol/10^{11} cells/min to mM/s, where platelet cytosolic volume was roughly estimated as 5 fL. Fluxes through key enzymes in each pathway were limited by maximum enzyme activity, calculated as:

$$Maximal\ enzyme\ activity = \frac{number\ of\ enzymes\ in\ one\ platelet \times turnover\ number\ of\ an\ enzyme}{platelet\ volume} \quad (1)$$

Hence, each pathway could be constrained by key enzyme activities: glycolysis is constrained by hexokinase (EC 2.7.1.1; v_{max} = 0.128 mM/s), respiration is constrained by pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4; v_{max} = 0.062 mM/s), isocitrate dehydrogenase (1.1.1.41), cytochrome c oxidase (EC 1.9.3.1; v_{max} = 0.145 mM/s) and specific acyl-CoA dehydrogenases (EC 1.3.8.1, EC 1.3.8.7, EC 1.3.8.8, EC1.3.8.9; v_{max} = 5.5 μM/s). The numbers of enzymes per platelet were from proteomic data [24] and enzyme turnover numbers were from human enzyme izoforms from BRENDA database (http://www.brenda-enzymes.org/).

2.2. Physiologically based human blood glucose kinetics

The paper of Markakis et al. [19] introduced a mathematical model consisting of 5 differential equations and representing the insulin – glucose dynamics of healthy subjects as well as Type 1 and Type 2 diabetics, with appropriate adjustment in its parameters.

Here we modified the model so that there is only one parameter describing blood glucose levels instead of two.

The equations are as follows:

$$\frac{dI}{dt} = -\gamma_I \cdot I + \beta \cdot i(G),\ i(G) = \begin{cases} 0,\ G < It \\ G - It,\ G \geq It \end{cases} \quad (2)$$

$$\frac{dN}{dt} = -\gamma_N \cdot N + \alpha \cdot n(G),\ n(G) = \begin{cases} Nt - G,\ G < Nt \\ 0,\ G \geq Nt \end{cases} \quad (3)$$

$$\frac{dx}{dt} = -p_1 \cdot x + p_2 \cdot I, \quad (4)$$

$$\frac{dG}{dt} = -p_3 \cdot G - p_4 \cdot x \cdot G + p_5 \cdot N, \quad (5)$$

where I is the blood insulin concentration in mU/l, N – blood glucagon concentration in ng/l (, x – variable indicating insulin action (min^{-1}), G – blood glucose concentration in mg/dl. Initial values for the variables were taken from [19]: I_0 = 15 mU/l, N_0 = 75 ng/l and x_0 = 0 min^{-1}, with exception for initial blood glucose concentration, which was taken [25] to be 100 mg/dl.

The model describes dynamics of glucose concentration and its main hormonal regulators and thus could be used to model blood glucose levels during day/night cycle. Figure S2 (Supplemental file 2) shows the scheme of glucose-insulin-glucagon relationships implemented in the model. The parameter values of the model are given in Table 1. Because we have modified the model (two equations for glucose are merged into one), some parameters needed adjustment. The parameter p_4 and hormone thresholds (Table 1) were adjusted by means of evolutionary programming algorithm [26,27] to describe glucose and insulin data for healthy subjects from [25].

2.3. PBPK/PD model with platelet metabolism

To unite the FBA and PBPK models we utilized principles from [25], when an organ is included in the model by two enzymes: a glucose transporter (GLUT) and a hexokinase (HK). The glucose, as an energy source, may be transported into the platelet mainly by GLUT3 transporters, those are present in plasmatic (15%) and intracellular alpha-granule (85%) membranes [28] and thus provide enhanced glucose flux after activation. As we assume all platelets to be in quiescent state, the turnover rate of GLUT3 was calculated as 15% of 1000 s^{-1} [25]. The GLUT3 and HK were considered to follow Michaelis-Menten kinetics with K_m 1.5 mM and 0.2 mM, respectively [25,28]. Thus the equation (5) was modified as follows:

$$\frac{dG}{dt} = -p_3 \cdot G - p_4 \cdot x \cdot G + p_5 \cdot N - \frac{V_{PLT}}{V_{Blood}} \frac{V_{max} \cdot f_{GLUT3} \cdot G}{K_M + G} \quad (6)$$

where V_{max} was calculated from eq. (1) for GLUT3 and f_{GLUT3} is the plasmatic membrane fraction of the transporters. The flux of glucose-6-phosphate (G6P) production resulting from HK could be considered as stationary input flux in the platelet FBA model (p. 2.1.). The

two pathways were assumed for G6P utilization, it could be used in glycogen production or in glycolysis. The linear relationship between the latter flux and platelet ATP production obtained from the flux model was incorporated in the PBPK model as a linear equation. The ATP production flux was fixed on 0.2 mM/s, the value obtained for quiescent platelets in the FBA model (see Results 3.1).

Table 1. Parameters of the PBPK model

Param.	Units	Value in [19]	Value	Physiological description	Ref.
γ_I	min^{-1}	0.42	0.42	Insulin clearance from blood	[19]
β	$\frac{mU/l}{mg/dl} min^{-1}$	0.106	0.106	Insulin production rate	[19]
It	mg/dl	103	60	Insulin threshold (minimum blood glucose concentration value for insulin production)	tuned
γ_N	min^{-1}	$5.8 \cdot 10^{-4}$	$5.8 \cdot 10^{-4}$	Glucagon clearance from blood	[19]
α	$\frac{ng/l}{mg/dl} min^{-1}$	0.0037	0.0037	Glucagon production rate	[19]
Nt	mg/dl	83	110	Glucagon threshold (maximum blood glucose concentration value for glucagon production)	tuned
p_1	min^{-1}	0.075	0.075	Insulin action decrease	[19]
p_2	$\frac{min^{-2}}{mU/l}$	$1.3 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$	Insulin action increase p_1 and p_2 together ensure the observed experimentally delay in insulin action after insulin production	[19]
p_3	min^{-1}	0.022	0.022	Glucose clearance from blood	[19]
p_4	nd	0.04	0.1	Glucose clearance provided by the insulin action	tuned
p_5	$\frac{mg/dl}{ng/l} min^{-1}$	0.016	0.016	Glucose production in the liver provided by glucagon action	[19]

2.4. Software

The linear programming problem was solved by using the GNU Linear Programming Kit [29]. The PBPK/PD model was analyzed using COPASI software [30].

3. Results and Discussion

3.1. Flux Balance Analysis of human platelet metabolism

3.1.1. *Energy metabolism on glucose*

The first and most common pathway of energetic metabolism is anaerobic glycolysis, when glucose obtained from the medium or from the internal storage (glycogen) is converted into lactate. The calculation of maximum platelet ATP production from the known glucose uptake flux is straightforward and doesn't need FBA model. From our calculations in case of anaerobic glycolysis upper bound for ATP production rate is 0.26 mM/s for glucose consumption rate (0,128 mM/s) determined by maximal hexokinase activity. Similarly anaerobic glycogenolysis gives 0.20 mM/s of ATP for the upper limit on glycogen consumption flux 66 μM/s [13].

Firstly we have analyzed experimental data for platelet suspensions without fatty acids or other than glucose substrates in the medium (Table 2). Each column of the table is an FBA calculation performed for an experimental study, with experimental data shown in parentheses and calculated data given without them. The columns are grouped into three types depending on the experimental conditions and design. The first group of works [3,5,9] includes measured lactate production and oxygen consumption rates in platelet. We assume that all oxygen goes into glucose oxidation. It should be noted that in work of Ravi et al. [3] lactate production was not directly measured, here it was recalculated from acidification of the medium according to works of Akkerman et al. [5,31]. From works of Akkerman's [12,13] estimation of fluxes in the glycogen metabolism can be included. The second group of works [11,32] includes measured rates of glucose, glycogen consumption, lactate production and glucose oxidation. Note that in Doery et al. [32] measured lactate production rate was 0.128 mM/s and does not correspond to glucose and glycogen consumption rates (0.055 and 0.032 mM/s, respectively) and glucose oxidation rate 0.535 μM/s. Therefore, we do not use lactate production rate as a constraint. In the study of Guppy et al. [8] rates of glucose, oxygen consumption, lactate production and glucose oxidation were measured, but total consumed oxygen is ten times the one needed for glucose, so it was not used as a constraint in the model.

Calculated ATP production for glucose oxidation varies from 0.1 to 0.66 mM/s depending on glucose and oxygen fluxes (Table 2). The contribution of glucose -> lactate pathway gives 21-88 % of ATP flux with 69-99% of glucose going into lactate.

Table 2. Main fluxes in the FBA of platelet *.

	W/o free fatty acids as fuels							With free fatty acids as fuels		
Group #/ fixed fluxes	**1 / lactate+oxygen**			**lactate+ oxygen + glycogen**	**2/ glucose + lactate + glycogen + CO_2**		**glucose + lactate + + CO_2**	**3/ lactate + CO_2+FFA**		
Reference	[9]	[3]	[5]	[12,13]	[11]	[2]	[8]	[2]	[7]	[11]
Glucose consumption	0.0175	0.0678	0.1280	0.0871	(0.0270)	(0.0550)	(0.0430)	(0.0300)	0.0437	(0.0270)
Glycogen consumption	-	-	0.0546	(0.0110)	(0.0.010)	(0.0320)	0.0116	(0.0220)	-	(0.0010)
Oxygen consumption	(0.0130)	(0.105)	(0.0330)	(0.0330)	0.0149	0.0056	0.0059	0.0093	0.0234	0.0264
Lactate production	(0.0300)	(0.0938)	(0.3520	(0.1830)	(0.0518)	(0.1729)	(0.1080)	(0.1032)	(0.0866)	(0.0518)
Glucose oxidation	0.0026	0.0210	0.0066	0.0066	(0.0021)	(0.000535)	(0.000625)	(0.0004)	(0.00044)	(0.0021)
FFA oxidation	-	-	-	-	-	-	-	(0.0003)	(0.0009)	(0.0006)
ATP production	0.10	0.66	0.58	0.37	0.13	0.23	0.15	0.17	0.20	0.18
Respiration, % [#]	65 / 15	79 / 31	28 / 4	44 / 7	57 / 8	12 / 1	19 / 1	26 / 1	56 / 1	70 / 8
Glycolysis & glycogenolysis, % [##]	35 / 85	21 / 69	72 / 96	56 / 93	43 / 92	88 / 99	81 / 99	74 / 99	45 / 99	30 / 92

* Values are expressed in mM/s. Values in parantheses were fixed corresponding to experimentally measured fluxes.

[#] Fraction of respiration in ATP production/ glucose utilization

[##] Fraction of glucose and glycogen consumption in ATP production/ glucose utilization.

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As could be seen from Table 2, measured oxygen fluxes are one [5,9] to two [3] orders of magnitude greater than the flux directed to glucose oxidation. It may be caused by some measurement errors of Clark's electrodes; thus we assumed that measurements of $^{14}CO_2$ evolved from labeled 6-^{14}C-glucose are more accurate. Then the fraction of oxidized glucose is 1-8 % instead of 15-31% and glycolysis from glucose to lactate makes the greatest contribution to the ATP production. Note that even in quiescent platelet the hexokinase enzyme works at 20-60% of its maximal velocity.

The experimental data on platelet glycogen consumption vary greatly in the literature (Table 2). It is interesting to note that glycogen used as a substrate approximately may be depleted in 3 days [11] or in 1.5-7 hours [5]. Thus, it can disprove the hypothesis that platelet do not use glycogen in a quiescent state.

According to these results, it is important to take into account fatty acids oxidation to make more accurate estimation of platelet energy metabolism.

3.1.2. *Energy metabolism on glucose and fatty acids*

Fatty acids oxidation was measured in several works [2,7] through labeled 6-^{14}C-palmitate. We also include in the analysis data from [11], where platelet rich plasma with fatty acids was used instead of washed platelets. As could be seen from Table 2 addition of palmitate oxidation does not vary ATP production rate greatly. Contribution of anaerobic glycolysis/glycogenolysis still exceeds contribution of respiration to energy metabolism.

Like other cells, platelets have stored fatty acids. We can estimate endogenous FFA oxidation from study with etomixir [3], where approximately 15% of oxygen is directed on endogenous FFA oxidation. Yet even addition of endogenous FFA oxidation at 900 nM/s does not change the situation.

Thus, it may be concluded that glycolysis is a principal source to platelet energy production, while the importance of oxidative metabolism is controversial. Sum of glucose, exogenous and endogenous fatty acids oxidation may mainly contribute to ATP production, but glucose is not the major fuel for these oxidative pathways.

On average, ATP production flux in quiescent platelets in glucose medium can be estimated as 0.2-0.3 mM/s with 99% of glucose going into lactate.

In the following section we test the hypothesis that stored fuels, mainly glycogen, can be used by platelets under "tight" conditions of low glucose in the medium.

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3.2. Physiologically based pharmacokinetics/pharmacodynamics

3.2.1. Analysis of PBPK model with platelets

To investigate platelet energy capabilities we have positioned a model of quiescent platelet described by stationary energy fluxes into a mathematical model of human daily blood glucose turnover. The physiologically based pharmacokinetics (PBPK) model was adapted from [19], where blood glucose levels are expressed in mg/dl (for glucose, 1 mM ≈ 18 mg/dl). First, the model was tuned to describe experimental data on average human glucose dynamics from [25] (Fig. 1). At time intervals (200, 280), (950, 1050) and (1280, 1360) min blood glucose input flux at 5 mg/dl/min was added to the model. The final model parameters are given in Table 1. It should be noted here that the experimental glucose time course is for an individual donor and thus the parameters of the model were assessed for this particular donor and may differ from generally accepted parameters.

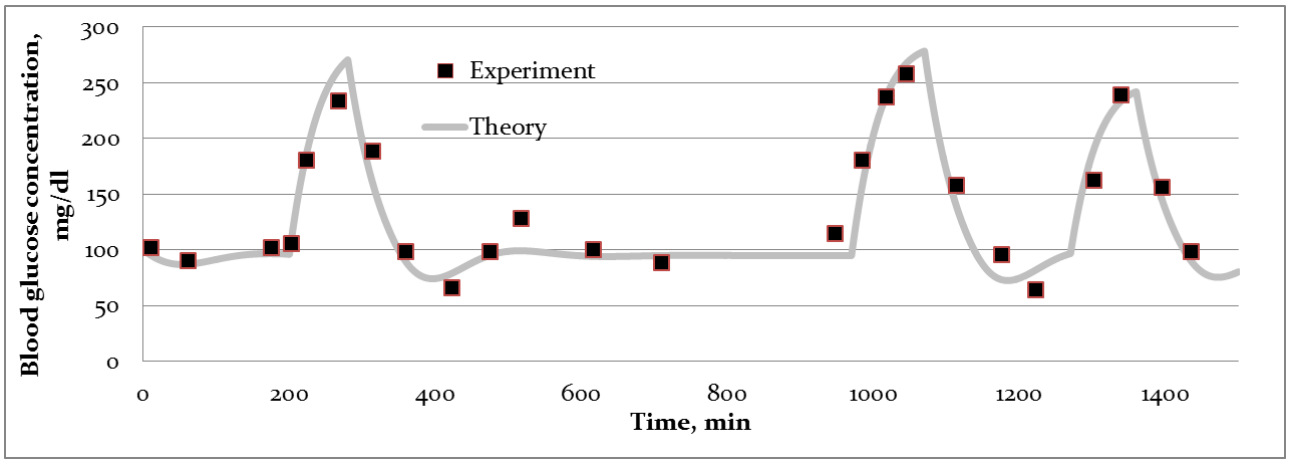

Figure 1. Blood glucose level in the PBPK model in comparison with experimental data [25]. At time intervals (200, 280), (950, 1050) and (1280, 1360) min blood glucose input flux at 5 mg/dl/min was added to the model.

The influence of blood platelets even with maximal glucose consumption rate of 0.128 mM/s at normal blood glucose level (100 mg/dl) equals 0.01 mg/dl/min and is negligible compared to normal fasting blood glucose turnover rate (155 mg/dl/min) [33].

Platelet glucose consumption fluxes (approx. 50-100 μM/s) are far larger than those for red blood cells and are approaching those for respirating white blood cells (50 μM/s [34]), yet, their relative volume is too small to have influence on whole organism glucose turnover.

3.2.2. Analysis of platelet metabolism dynamics in human blood

Normal human fasting glucose levels vary from 70 to 100 mg/dl (or 3.5-5.5 mM) with maximum levels of 360 mg/dl after meal and minimum of 50 mg/dl during starvation [35]. For platelet glucose transporter Km = 27 mg/dl, that is below the blood glucose minimum level, so the platelet glucose flux depends mainly on the number of GLUT3 transporters on the platelet membrane. Yet as was discussed in previous section (3.1) platelet metabolism depends mainly on glucose input. We investigated platelet behavior in conditions of rather tight diet with one short period of high blood glucose influx (10 mg/dl) at time interval (100,120) min and one long period of low blood glucose influx (1 mg/dl at time interval (500,700) min (Fig. 2). Under such diet blood glucose levels vary from 75 to 300 mg/dl (Fig. 2A). Platelet metabolism was investigated for 3 values of the plasmatic membrane fraction of GLUT3, 10% (Fig. 2B), 12% (Fig. 2C) and 15% (Fig. 2D).

The platelet glycogen storage depends on glucose level and number of glucose transporters with high sensitivity. As switch between glycogen storage and glycogen consumption could be regulated by the activity of GLUT3 and HK, it is probable that in real cells it is regulated depending on ATP production and glucose-6-phosphate flux.

3.2.3. Sensitivity analysis of platelet glycogen storage

Obviously the parameters influencing platelet energy storage are not limited to GLUT3 and blood glucose levels. We performed sensitivity analysis of the parameters of the PKPD model with platelets to find other candidates. The sensitivity parameter tells how sensitive the output is to a perturbation of the input or in the parameters of the model [36]. As the output, we have chosen the concentration of platelet glycogen at 1000 min of glucose course described in Fig. 2. The detailed results of sensitivity analysis are shown in Table 3. It appeared that platelet GLUT3 and ATP production are indeed the parameters with highest sensitivity values. Interestingly, platelet glycogen level is sensitive to the parameters of glucagon dynamics two orders of magnitude more than to the parameters of insulin dynamics. In summary, sensitivity analysis shows that platelet glycogen level is more sensitive to platelet parameters than to blood glucose levels.

Table 3. Sensitivity analysis of the model (scaled sensitivities of the parameters)

Parameter	Sensitivity
γ_I	$3.6 \cdot 10^{-4}$
β	$-3.9 \cdot 10^{-4}$
It, p_2, p_4	$3.8 \cdot 10^{-4}$
γ_N	-0.02
α	0.02
Nt	0.13
p_1	$3.4 \cdot 10^{-4}$
p_3	-0.03
GLUT3 plasmatic membrane fraction	0.67
GLUT3 Km	-0.14
HK Km	$-9 \cdot 10^{-4}$
ATP production	-0.52

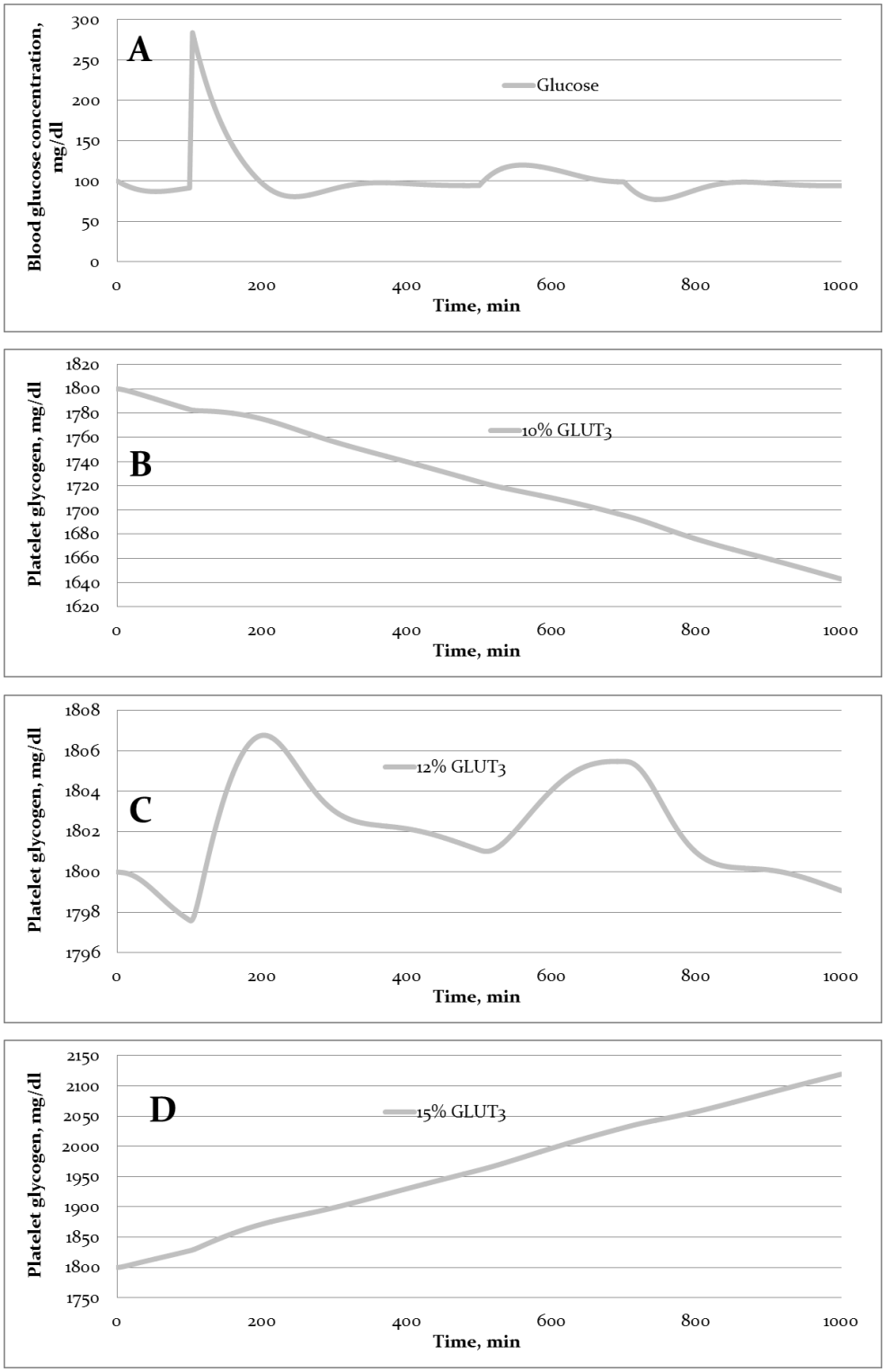

Fig. 2. Dependence of blood glucose and platelet glycogen on blood glucose influx. The calculated time-courses of the levels of blood glucose (A) and platelet glycogen (B-D) for the model with two "meals" are given. The "meal" is the blood glucose input flux. The first, 10 mg/dl, was at time interval (100,120) min and the second, 1 mg/ml, was at (500,700) min. The initial platelet glycogen level was taken to be 1800 mg/dl (corresponds to 50 μmole of glycosyl residues per 10^{11} cells). The platelet ATP production flux was fixed at 0.08 mM/s (minimal observed in the experiment ATP production from glucose). The plasmatic membrane fraction of GLUT3 varies: **(B)** 15%, **(C)** 12% and **(D)** 10%.

Conclusions

Here we have applied Flux Balance Analysis and Pharmacokinetics/Pharmacodynamics modeling to analyze platelet functioning in human physiology. The aim of our study was to bring together scattered data on quiescent platelet metabolism, and propose which of the proposed in the literature [2,3,7] metabolic pathways (anaerobic glycolysis, oxidative phosphorylation or fatty acid oxidation) is responsible for platelet ATP production in quiescent state. From FBA it could be concluded that platelet uses both glycolysis and respiration for ATP production. In comparison with respiration, glycolysis (hexokinase) works close to its maximum and most of glucose is converted to lactate. Estimations allow us to make a conclusion that in quiescent state glucose prevails in ATP production over exogenous and endogenous fatty acids and other endogenous fuels oxidation. The role of glycogenolysis in platelet metabolism could not be assessed from FBA, as it depends on platelet glucose and oxygen consumption fluxes. The glucose consumption flux is determined by platelet glucose transporter, GLUT3, and hexokinase enzyme. The latter already works close to its limit. The former depends on the number of GLUT3 in platelet membrane and blood glucose concentration. The proposed PBPK/PD model allows the assessment of the mutual influence of platelet metabolism and blood glucose levels. We find that platelet metabolism, despite high glucose consumption rates, does not much influence blood glucose levels due to small relative platelet volume. The blood glucose influence on platelet metabolism strongly depends on fraction of GLUT3 in platelet plasmatic membrane. Platelet glycogen could be continuously stored for 15% of GLUT3, continuously depleted for 10% of GLUT3 or synthesized after meal and depleted after fasting for 11-14%. These finding suggest that platelets contain big amounts of the stored glycogen for a case of a prolonged fast rarely met in the contemporary world.

Acknowledgements

The study was supported by Russian Foundation for Basic Research grants 15-29-01313, 16-34-00651 and 15-34-70009, and by the Russian President Grants MD-6347.2015.4 and MK-5879.2016.4.

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