Supporting information for „Systems analysis of metabolism in buffy coat and apheresis derived platelets during storage“

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Protein extraction and processing.

Method 1: The PC samples were centrifuged (1,000 x g; 4°C; 5 min) and the supernatant discarded. The cell pellets were washed twice using 5 mL of phosphate-buffered saline and lysed with 5 mL of lysis buffer (0.05 mM Tris/HCl, 1 mM EDTA, 125 mM CaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.45 sodium fluoride, 0.45 mM ß-glycerophosphate, 0.09 mM sodium orthovanadate and protease inhibitor cocktail (cOmplete™ Protease Inhibitor Cocktail, Sigma-Aldrich St.Louis, MO). Samples were sonicated on ice for three rounds of 10 second burst and the cell lysates centrifuged (20,817 x g; 4°C; 20 min), and the supernatant collected. The protein concentration was measured using the BCA method (Thermo Scientific Pierce BCA protein assay kit, USA).

Method 2: The PC samples were centrifuged (1,000 x g; 4°C; 5 min) and the supernatant discarded. The cell pellets were washed twice using 5 mL of phosphate-buffered saline and lysed with 5 mL of

lysis buffer (4% Sodium dodecyl sulfate (SDS) in 100mM Tris). The samples were kept on ice for 10 minutes, and transferred to a 1.5ml Eppendorf tube. After five freeze/thaw cycles (-80°C/room temperature), the samples were centrifuged at 14,000 rpm for 20 minutes at 4˚C. The supernatants were collected and aliquoted into new vials and stored at -80˚C. The protein concentration was measured using the BCA method (Thermo Scientific Pierce BCA protein assay kit, USA).

Model refinement. To address gaps in the iAT-PLT-636 model, 7 metabolic reactions (KGMALtm, ASPGLUm, CITtam, ACSm, PPAm, ADK1m, RPE) were added, all of which have been detected in proteomic experiments (1). Also added to the model were the electroneutral diffusion of acetate across the mitochondrial membrane (ACt2m) on the grounds that the unionized form acetic acid can diffuse over lipid membranes. Further 4 reactions (EX_k(e), ASNS1, ASNtN1, NH4D, sink_orn[c]) where added to resolve infeasibility of the model. (Table S3.) 12 reactions were removed from the iAT-PLT-636 model either due to a lack of evidence for the reactions (ACCOAtm) and/or to eliminate internal loops (FBP, SUCOASm, SBTR, SBTD_D2, PFK26, GALT, UGLT, DPGM, DPGase, PPDOx, PPDOy, SO4t4_2) (see table S4.)

Energy partition definitions. Net ATP is calculated by adding the mean fluxes of the reactions: ATP synthase (ATPS4m), Succinate--CoA ligase (GDP-forming) (SUCOAS1m) and Pyruvate kinase (PYK), and subtracting 2.25 times the mean flux of the Acetyl-CoA synthetase (ACSm) reaction. The percentage of ATP produced in the cytosol via glycolysis is the ratio of the mean flux of PYK and the Net ATP. The mitochondrial net ATP production is 100%-(cytosolic net ATP production). To calculate the partition based on P/O ratios the mitochondrial ATP production is defined as 2 x oxygen consumption x P/O ratio. Or 2 x mean flux through oxygen transport reaction (O2t) x 2.25 (the P/O ratio). Glycolytic ATP production is equal to L-lactate secretion i.e. 1 x L-lactate transport reaction (L_LACt2r). The total energy production is then the mitochondrial ATP production plus glycolytic ATP production.

Table S1: Detailed summary of the data sets used in the study.

	AP-PC (baseline)	**BC-PC (baseline)**	**AP-PC (^{13}C labelled citrate)**	**BC-PC (proteomics)**
Reference	(2)	(3)	This study	This study
Number of subjects	8	40	2	15
Number of bags	8	8	2	3
Sampling days	0,1,3,4,5,6,7,10	1,2,3,4,5,6,7,10	0,1,2,3,4,5,6,7,10	1,3,6
Definition of stage 1	Days 1,3	Days 1,2,3	n.a.	n.a.
Definition of stage 2	Days 4,5,6	Days 4,5,6	n.a.	n.a.
Number of extracellular metabolites quantified	53	63	n.a.	n.a.
Number of extracellular metabolites used	18	19	n.a.	n.a.
Number of hematological parameters used	1	1	n.a.	n.a.

n.a.: not available.

Table S2: List of extracellular metabolites and blood gas parameters included in the study.

AP-PC (baseline)	**BC-PC (baseline)**
PLT count	PLT count
Acetate	Acetate
Citrate	Citrate
Glucose	Glucose
Lactate	Lactate
Glutamine	Glutamine
Arginine	Glutamate
Asparagine	Arginine
Hypoxanthine	Asparagine
Inosine	Hypoxanthine
Proline	Inosine
Succinate	Proline
Urate	Succinate
Malate	Urate
Xanthine	Malate
Oxoproline	Xanthine
Citrulline	Oxoproline
Fructose	Citrulline
Aconitate	Fumarate
	Aconitate

Table S3. Reactions added to iAT-PLT-636. The proteomic evidence comes from 1: this study, 2 = PlateletWeb (1), 3 = Proteomic study by Maaike Rijkers et al. (4). The metabolic evidence is based on the ^{13}C labelling data from this study.

Reaction name	Reaction Formula	Description	Gene	Evidence
EX_k(e)	k[e] <=>	Potassium exchange	n.a.	n.a.
KGMALtm	akg[m] + mal_L[c] <=> akg[c] + mal_L[m]	α-ketoglutarate/malate antiporter	SLC25A11	Proteomic (1,2,3)
ASPGLUm	asp_L[m] + glu_L[c] + h[c] <=> asp_L[c] + glu_L[m] + h[m]	aspartate/glutamate antiporter	SLC25A13	Proteomic (1)
CITtam	cit[c] + h[c] + mal_L[m] <=> cit[m] + h[m] + mal_L[c]	citrate/malate antiporter	SLC25A1	Proteomic (1,2,3)
PEPtam	cit[c] + h[c] + pep[m] <=> cit[m] + h[m] + pep[c]	citrate/PEP antiporter	SLC25A1	Proteomic (1,2,3)
ACt2m	h[c] + ac[c] <=> h[m] + ac[m]	acetate mitochondrial transport	n.a.	n.a.
ACSm	atp[m] + coa[m] + ac[m] -> accoa[m] + amp[m] + ppi[m]	acetyl-coA synthetase	ACSS1	Proteomic (1,2,3)
PPAm	h2o[m] + ppi[m] -> h[m] + 2 pi[m]	inorganic diphosphatase, mitochondrial	PPA2	Proteomic (1,2,3)
NH4D	nh4[c] <=> nh4[m]	Ammonia mitochondrial diffusion	n.a.	n.a.
ASNtN1	asn_L[e] + h[c] + 2 na1[e] <==> asn_L[c] + h[e] + 2 na1[c]	Asparagine transport	SLC38A5	n.a.
Lac_D_Transport	lac_D[c] + h[c] <=> lac_D[e] + h[e]	D-lactate transport	SLC16A7/ SLC16A3	Proteomic (1,2)
EX_LacD_e	lac_D[e] <=>	D-lactate exchange	n.a.	n.a.
PPCKm	gtp[m] + oaa[m] <=> gdp[m] + co2[m] + pep[m]	Phosphoenolpyruvate carboxylase kinase 2	PCK2	Proteomic (1,2,3)
ADK1m	atp[m] + amp[m] <=> 2 adp[m]	adenylate kinase, mitochondrial	AK2	Proteomic (1,2,3)
RPE	ru5p_D[c] <==> xu5p_D[c]	Ribulose 5-phosphate 3-epimerase	RPE	Proteomic (1,2,3)
ASNS1	asp_L[c] + atp[c] + gln_L[c] + h2o[c] --> amp[c] + asn_L[c] + glu_L[c] + h[c] + ppi[c]	Asparagine synthetase (glutamine hydrolyzing)	ASNS	n.a.
sink_orn[c]	orn[c] <=>	Ornithine sink reaction	n.a.	n.a.
CBPS	2 atp[c] + gln_L[c] + h2o[c] + hco3[c] -> 2 adp[c] + cbp[c] + glu_L[c] + 2 h[c] + pi[c]	carbamoyl synthetase 2	CAD	Proteomic (1,2,3)
ASPCT	asp_L[c] + cbp[c] -> cbasp[c] + h[c] + pi[c]	Aspartate carbamoyltransferase	CAD	Proteomic (1,2,3)
DHORTS	dhor_S[c] + h2o[c] <=> cbasp[c] + h[c]	Dihydroorotase	CAD	Proteomic (1,2,3)
DHORD_NAD	dhor_S[c] + nad[c] <=> orot[c] + nadh[c]	Dihydoorotic acid dehydrogenase	DHODH	Proteomic (2)
ORPT	orot5p[c] + ppi[c] <=> orot[c] + prpp[c]	Orotate phosphoribosyltransferase	UMPS	Proteomic (1,2,3)
OMPDC	orot5p[c] + h[c] -> co2[c] + ump[c]	Orotidine-5'-phosphate decarboxylase	UMPS	Proteomic (1,2,3)

EX_acon(e)	acon[e] <=>	Aconate exchange	n.a.	n.a.
acontT2	acon[e] <=> acon[c]	Aconate transport	n.a.	metabolomic
ACONT1	cit[c] <=> acon[c]	Aconitase	ICDHy	metabolomic
ACONT2	acon[c] <=> icit[c]	Aconitase	ICDHy	metabolomic

n.a.: Not available

Table S4. Reaction removed from iAT-PLT-636.

Reaction name	Reaction Formula	Description	Gene(s)
SUCOASm	atp[m] + coa[m] + succ[m] <=> pi[m] + succoa[m] + adp[m]	Succinate--CoA ligase (ADP-forming)	SUCLG1 and SUCLA2
PFK26	f6p[c] + atp[c] -> h[c] + adp[c] + f26bp[c]	6-phosphofructo-2-kinase	PFKFB2 or PFKFB3 or PFKFB4 or PFKFB1
GALT	utp[c] + gal1p[c] + h[c] <=> udpgal[c] + ppi[c]	galactose-1-phosphate uridylyltransferase	GALT
UGLT	gal1p[c] + udpg[c] <=> udpgal[c] + g1p[c]	UDPglucose--hexose-1-phosphate uridylyltransferase	GALT
FBP	h2o[c] + fdp[c] -> f6p[c] + pi[c]	fructose-bisphosphatase	FBP1 or FBP2
ACCOAtm	accoa[c] -> accoa[m]	Acetyl-coA tranporter	n.a.
PPDOx	lald_D[c] + nadh[c] + h[c] <=> nad[c] + 12ppd_R[c]	Propane-1,2-diol:NAD+ 1-oxidoreductase	AKR7A2
PPDOy	lald_D[c] + h[c] + nadph[c] -> nadp[c] + 12ppd_R[c]	Propane-1,2-diol:NADP+ 1-oxidoreductase	AKR7A2 or AKR1A1 or AKR1B1
SO4t4_2	2 na1[e] + so4[e] <=> 2 na1[c] + so4[c]	Sulfate transport	SLC13A4
LDH_D	nad[c] + lac_D[c] <=> pyr[c] + nadh[c] + h[c]	D-lactate dehydrogenase	LDHD
ACONT	cit[c] <=> icit[c]	Aconitase	
MALSO3tm	mal_L[c] + so3[m] <=> mal_L[m] + so3[c]	Malate:sulfite antiport, mitochondrial	SLC25A10
MALTSULtm	mal_L[c] + tsul[m] <=> mal_L[m] + tsul[c]	Malate:thiosulfate antiport, mitochondrial	SLC25A10
MALtm	malate transport, mitochondrial	mal_L[c] + pi[m] <=> mal_L[m] + pi[c]	SLC25A10

n.a.: Not available

Table S5: Metabolite uptake and secretion rates in mmol/day/10^{12} platelets used as constraints at each stage and condition. Negative values denote metabolite uptake and positivevalues secretion. The samples in the apheresis and buffy coat sets were analyzed by UPLC-MS in two separate batches and batch effects were taken into account by using the ComBat algorithm (5).

Metabolite	Condition	Stage 1	Stage 2	Stage 3
Glucose	BC-PC	-0.46	-0.62	-0.70
	AP-PC	-0.64	-0.65	-0.32
Lactate	BC-PC	0.90	1.07	1.27
	AP-PC	1.19	1.11	0.57
Acetate	BC-PC	-0.95	-1.20	-0.64
	AP-PC	-0.82	-0.80	-0.42
Glutamine	BC-PC	-0.027	-0.038	-0.029
	AP-PC	-0.0072	-0.0086	-0.0054
Glutamate	BC-PC	0.0004	-0.065	-0.0030
	AP-PC	0.0025	-0.0046	0.00051
Citrate	BC-PC	-0.0075	0.034	-0.019
	AP-PC	-0.041	0.0097	-0.034
Arginine	BC-PC	0.0036	0.0044	0.0088
	AP-PC	0.0035	-0.00043	0.0022
Asparagine	BC-PC	-0.0022	-0.00055	0
	AP-PC	0.00073	0.00047	0.00011
Hypoxanthine	BC-PC	0.018	0.012	0.011
	AP-PC	0.0076	0.0024	0.0048
Inosine	BC-PC	0.00023	0.000011	-0.0000058
	AP-PC	0.000091	0	0.000056
Proline	BC-PC	0.0013	0.015	0.0084
	AP-PC	0.010	0.0026	0.0043
Succinate	BC-PC	0.0015	0.00046	0.0012
	AP-PC	0.0015	-0.00020	-0.000016
Urate	BC-PC	0.0023	-0.00092	-0.00080
	AP-PC	-0.0028	-0.0085	-0.00021
Malate	BC-PC	0.0033	-0.00087	0.0074
	AP-PC	0.0024	0.0026	0.0047
Xanthine	BC-PC	0.0022	0.0026	0.0025
	AP-PC	0.0026	0.0017	0.0027
Oxoproline	BC-PC	-0.0064	-0.0037	-0.0060
	AP-PC	-0.0028	-0.00088	-0.0021
Citrulline	BC-PC	0.0011	0.00044	0.00057
	AP-PC	0.00054	-0.0023	-0.00024
Fumarate	BC-PC	0.038	0.0060	0.075
	AP-PC	0	0	0
Fructose	BC-PC	0	0	0
	AP-PC	-0.0034	-0.0040	-0.0028
Aconitate	BC-PC	0.0073	0.0047	0.0083
	AP-PC	0.0065	0.0042	0.0020

Table S6. Adjustment required to obtain feasible models at each condition and stage. To resolve infeaslable models the exchange fluxes were adjusted (relaxed) by altering the upper (u) and/or lower (l) bounds. A more detailed description can be seen in *Model constraints derived from metabolomics data.*

	Reaction name	Before Constraint Relaxation ([l, u])	After Constraint Relaxation ([l, u])
BC-PC			
Stage 1			
Rxn 1.	EX_glu_L(e)	[0.0004,0.0004]	[0.0000,0.0004]
Rxn 2.	EX_ile_L(e)	[0.0000,1000.0000]	[-0.0018,1000.0000]
Stage 2	-	-	-
Stage 3			
Rxn 1.	EX_ile_L(e)	[0.0000,1000.0000]	[-0.0237,1000.0000]
Rxn 3.	EX_5oxpro(e)	[0.0002,0.0002]	[0.0000,0.0002]
AP-PC			
Stage 1			
Rxn 1.	EX_gly(e)	[0.0000,1000.0000]	[-0.0001,1000.0000]
Rxn 2.	EX_glyb(e)	[0.0000,1000.0000]	[-0.0001,1000.0000]
Rxn 3.	EX_urate(e)	[-0.0028, -0.0028]	[-0.0028, -0.0013]
Rxn 4.	EX_ahcys(e)	[0.0000,1000.0000]	[-0.0001,1000.0000]
Stage 2			
Rxn 1.	EX_urate(e)	[-0.0085, -0.0085]	[-0.0085, -0.0008]
Rxn 2.	EX_arg_L(e)	[-0.0004, -0.0004]	[-0.0004, -0.0003]
Stage 3			
Rxn 1.	EX_gly(e)	[0.0000,1000.0000]	[-0.0000,1000.0000]
Rxn 2.	EX_glyb(e)	[0.0000,1000.0000]	[-0.0000,1000.0000]
Rxn 3.	EX_ahcys(e)	[0.0000,1000.0000]	[-0.0000,1000.0000]

Table S7. The relative contribution of metabolic pathways to ammonia production as predicted by the models. Data are expressed as mean ± standard deviation.

Pathway	Condition	Stage 1	Stage 2	Stage 3	Literature values	References
Glutamine degradation	BC-PC	69.1 % ± 1.5	80.5 % ± 2.8	80.8 % ± 3.5	n.a.	n.a.
	AP-PC	67.8 % ± 1.8	76.7 % ± 4.3	63.5 % ± 1.5		
Asparagine degradation	BC-PC	3.7 % ± 0.9	2.4 % ± 1.4	1.6 % ± 1.2	n.a.	n.a.
	AP-PC	1.2 % ± 0.8	2.9 % ± 2.1	1.0 % ± 0.7		
Purine degradation	BC-PC	29.0 % ± 0.4	14.3 % ± 1.0	17.6 % ± 3.2	n.a.	n.a.
	AP-PC	28.3 % ± 0.0	16.8 % ± 2.5	33.9 % ± 0.4		

Table S8. NADPH turnover rates and partition of total NADPH turnover. The turnover rates are reported in mmol/day/10^{12} platelets. The contribution of the main NADPH pathways and single reaction is reported as percentage of total NADPH turnover. *Abbreviations:* pentose phosphate pathway (PPP), isocitrate dehydrogenase (ICDH), malic enzyme (ME).

	Condition	Stage 1	Stage 2	Stage 3
Total NADPH turnover rate	BC-PC	0.098	0.498	0.105
	AP-PC	0.222	0.262	0.068
Cytosolic NADPH turnover rate	BC-PC	0.088	0.44	0.093
	AP-PC	0.199	0.236	0.060
Mitochondrial NADPH turnover rate	BC-PC	0.010	0.057	0.012
	AP-PC	0.022	0.026	0.008
PPP (cytosolic) % of total	BC-PC	18.1	27.9	26.9
	AP-PC	27.6	27.4	26.4
ICDH (cytosolic) % of total	BC-PC	67.2	55.7	57.5
	AP-PC	57.7	58.0	58.2
ICDH (mitochondrial) % of total	BC-PC	9.8	7.9	11.3
	AP-PC	8.8	7.8	6.1
ME (mitochondrial) % of total	BC-PC	0.0	3.5	0.0
	AP-PC	1.4	2.2	5.0
Other % of total	BC-PC	4.9	5.0	4.3
	AP-PC	4.5	2.8	4.3

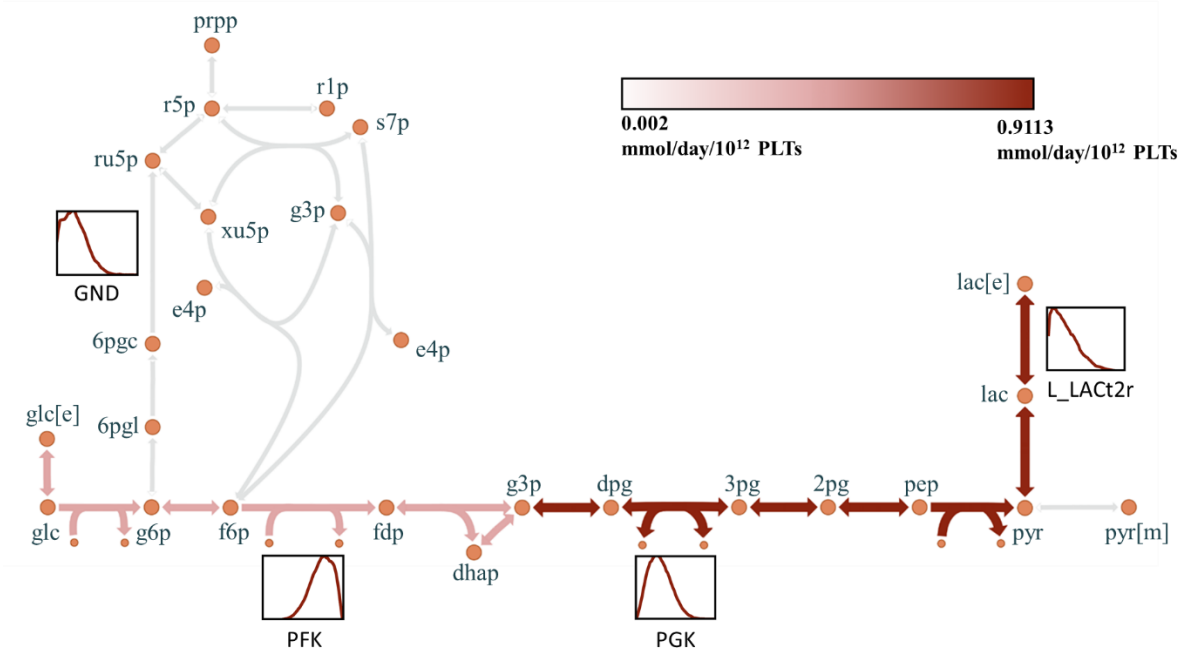

Figure S1: Flux predictions for the glycolytic and pentose phosphate pathways during days 1 – 3 (stage 1) in buffy coat units. Glucose is converted to pyruvate which is virtually all secreted as lactate with only a small part of the pyruvate transported to the mitochondria. The flux is partially diverted through the pentose phosphate pathway. Probability distributions are shown for selected reactions inside black boxes. The horizontal axis represents the magnitude of the flux and is between 0 and 1 mmol/day/10^{12} PLTs. The color indicates average flux value of probability distributions for that reaction. Abbreviation can be found in the BiGG database (http://bigg.ucsd.edu/).

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