

Fueling Platelets Where Does the Glucose Come From?

Sidney W. Whiteheart

There has long been interest in the metabolic pathways occurring in platelets and their relationship to function. Despite their reliance on crude analytic tools and inhibitors, early studies showed that platelets must produce ATP to maintain functionality.¹ Platelet activation leads to a significant increase in glucose uptake, glycolysis, and lactate production,²⁻⁵ suggesting that, in addition to oxidative phosphorylation, glycolysis is a significant producer of ATP to fuel platelet function. Electron microscopy studies clearly show that platelets contain glycogen granules, which could serve as a reservoir for energy production. Glucose can also be imported from the outside through 3 classes of facilitative transporters (glucose transporters [GLUTs], sodium GLUTs, and sugars will eventually be exported transporters).^{6,7} These proteins facilitate the transmembrane movement of glucose down a concentration gradient of either glucose or sodium.⁶ Platelets contain at least 2 members of the GLUT family of uniporters, GLUT1 and GLUT3.^{8,9} Although GLUT1 may account for basal uptake of glucose in resting cells, GLUT3 has drawn attention because it is mobilized from α -granules to the plasma membrane on platelet activation (Figure 1).^{4,5} In this issue, Fidler et al⁹ used a platelet-specific knockout of GLUT3 to probe its role and, thus, the roles of regulated glucose uptake, in platelet function.

See accompanying article on page 1628

GLUT3 was originally posited as a neuronal-specific GLUT that maintained cytoplasmic levels of the neuron's preferred fuel.¹⁰ Subsequently, it was detected on sperm, and studies of GLUT3 knockout mice showed it is globally required for embryogenesis.¹¹ GLUT3 is unique, having the lowest K_m and highest V_{max} of the 4 major GLUTs (1-4); thus, it has a high transport capacity even at lower glucose concentrations.¹² Localized to the plasma membrane in neurons and sperm, GLUT3 exists in an intracellular pool in certain blood cells, for example, lymphocytes, macrophages, neutrophils, and platelets.¹¹ It is from this intracellular pool that it is mobilized to the plasma membrane on activation,^{4,5} much like GLUT4 is mobilized in adipose and muscle cells on insulin treatment.

Fidler et al⁹ created mice lacking GLUT3 in platelets and megakaryocytes. Megakaryocytes from these mice showed no overt defects, and platelet numbers were unaffected, suggesting that GLUT3 was not required for platelet production. Although the mice had no defect in the tail bleeding assay of hemostasis, there were mild but significant defects in the autoimmune/inflammatory arthritis model and some protection in the collagen/epinephrine pulmonary embolism model. In vitro, GLUT3^{-/-} platelets produced normal microparticles in response to thrombin and collagen but were defective in spreading and clot retraction. Aggregation was also defective but only at low agonist concentrations, and the defects were resolved when agonist was increased. These data show a role for GLUT3 in platelet function but suggest that the transporter is not absolutely required because no assays showed a complete ablation of function.

Secretion from GLUT3^{-/-} platelets was only partially affected. ATP release and exposure of CD63 were unaffected but the release of PF4, IL1 α , and exposure of CD62 were, suggesting that release of dense granule cargo did not require GLUT3 but that α -granule content release did. Because dense granules are thought to be docked to the plasma membrane, they may not require extra ATP generation for membrane fusion.¹³ Ultrastructural analysis of α -granules showed that activated GLUT3^{-/-} platelets had more decondensed granules relative to wild type. Thus, membrane fusion occurred, but the cargo was not effectively dissolving or being extruded from the granules. This suggests that GLUT3 supplies the fuel for fusion pore expansion¹⁴ or an active extrusion of granule content. Alternatively, GLUT3 may transport polyol glucose into the concentrated granule cores to promote solubilization of α -granule contents. Future studies will be needed to address these points.

Secretion from the GLUT3^{-/-} platelets had a distinctive time course that suggested 2 sources of glucose may fuel different stages of α -granule secretion. Consistently, glycogen levels rapidly decreased at the initial time points, suggesting that glycogenolysis was important in the early stages of activation. To address this, Fidler et al⁹ blocked glycogen phosphorylase and slowed the rate of secretion but did not affect its final extent in wild-type mouse and human platelets. Similar treatment of GLUT3^{-/-} platelets decreased both the initial rate and extent of *P*-selectin exposure. These data imply a role for glycogenolysis in the early stages of platelet activation and glucose influx, via GLUT3, at later stages. Although these data show that differential mobilization of fuel sources is important for platelet function, a simple interpretation is complicated by the fact that GLUT3^{-/-} platelets had lower resting levels of glycogen.

Given that GLUT3 transports glucose bidirectionally in response to a glucose gradient, does GLUT3 on α -granules

From the Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY.

Correspondence to Sidney W. Whiteheart, PhD, Department of Molecular and Cellular Biochemistry, University of Kentucky, 741 S Limestone, Lexington, KY 40536. E-mail whitehe@uky.edu

(*Arterioscler Thromb Vasc Biol.* 2017;37:1592-1594.)

DOI: 10.1161/ATVBAHA.117.309841.)

© 2017 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.117.309841

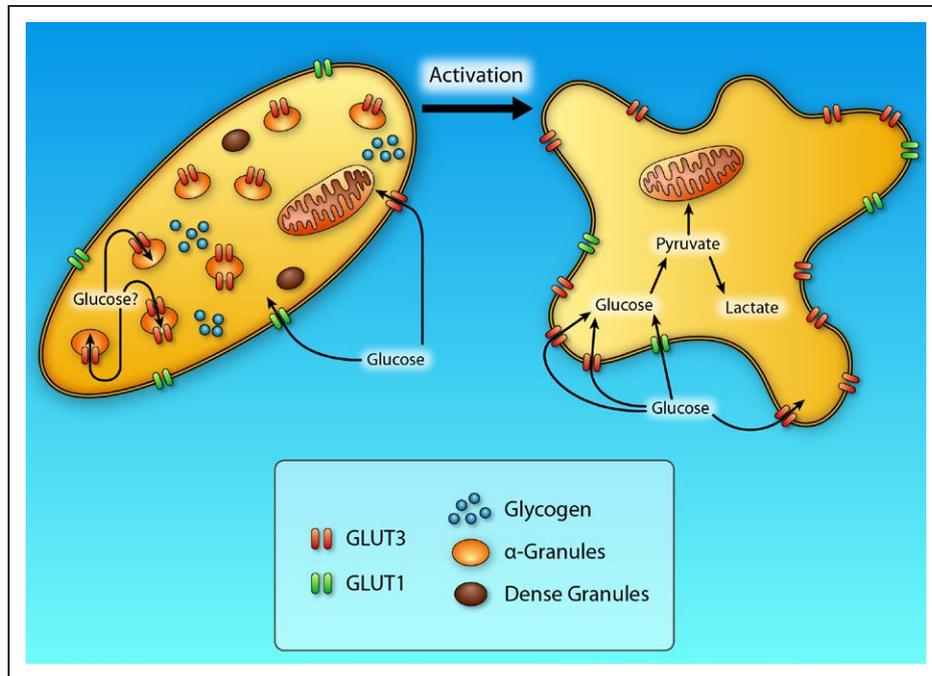


Figure 1. Mobilization of glucose transporters (GLUT3) in activated platelets. Although GLUT1 resides on the plasma membrane (PM), GLUT3 is present mainly in the α -granules of resting platelets and is mobilized to the PM on activation. There, GLUT3 promotes increased glucose influx which fuels the generation of ATP and, thus, platelet function, through oxidative phosphorylation in the mitochondria and glycolysis in the cytosol. Given the increase in glucose, much of the pyruvate produced by glycolysis in activated platelets seems to end up in lactate production.

have a role in resting platelets? Heijnen et al⁴ showed that $\approx 85\%$ of GLUT3 was on α -granules in resting cells. Although α -granules could simply be the depot for GLUT3, Fidler et al⁹ suggest GLUT3 might facilitate glycolysis and ATP generation in α -granules (Figure 1). Consistent with this concept, proteomics studies have detected glycolytic enzymes in platelet releasates.¹⁵ Fidler et al⁹ show some association of hexokinase and lactate dehydrogenase with a α -granule fraction, although the activity of lactate dehydrogenase in that fraction was low. Perhaps more compelling, the production of [¹³C] lactate in saponin-permeabilized, cytosol-depleted platelets decreased by 2.5-fold in the GLUT3^{-/-} platelets when fed [¹³C] glucose. However, because of difficulties in isolating intact α -granules, a true smoking gun demonstration that α -granules catalyze glycolysis is lacking. Future experiments in this area will assuredly be exciting. Does ATP generation truly occur inside α -granules and is that ATP contributing to platelet energetics? Alternatively, is that pool of ATP fueling intragranular phosphorylation or perhaps ecto-phosphorylation reactions outside the platelet?¹⁶ However, given the ATP released from dense granules, it is unclear how much more α -granules can contribute to the microenvironment around an activated platelet.

How does GLUT3 affect platelet function? Based on the data presented in Fidler et al,⁹ the answer may be subtle. GLUT3 does account for the stimulation-dependent influx of glucose seen in activated platelets, confirming previous work.^{4,5} This glucose influx is used to fuel spreading, clot retraction, and the sustained phase of α -granule secretion but perhaps not its initial steps. The enzymatic properties of GLUT3 make it ideal for efficiently transporting glucose when in a thrombus

core where diffusion is restricted.¹⁷ Thus, its mobilization to the surface offers a mechanism to fuel clot retraction and other late stages of thrombosis. In a broader sense, Fidler et al⁹ expand a new chapter in platelet energetics where the tools of metabolomics and genetically modified mice are used to ask specific questions about how platelets power thrombosis and hemostasis. Such future studies will yield important insights into normal platelet function and perhaps an understanding of the dysfunctions associated with metabolic pathologies, such as diabetes mellitus.

Sources of Funding

This work is supported by grants from the National Institutes of Health, National Heart, Lung, and Blood Institute (HL56652 and HL138179), from the American Heart Association Grant-in-Aid AHA16GRNT27620001, and a Veterans Affairs Merit Award to Dr Whiteheart.

Disclosures

None.

References

- Holmsen H. Platelet metabolism and activation. *Semin Hematol.* 1985;22:219–240.
- Akkerman JW, Holmsen H. Interrelationships among platelet responses: studies on the burst in proton liberation, lactate production, and oxygen uptake during platelet aggregation and Ca²⁺ secretion. *Blood.* 1981;57:956–966.
- Detwiler TC, Zivkovic RV. Control of energy metabolism in platelets. A comparison of aerobic and anaerobic metabolism in washed rat platelets. *Biochim Biophys Acta.* 1970;197:117–126.
- Heijnen HF, Oorschot V, Sixma JJ, Slot JW, James DE. Thrombin stimulates glucose transport in human platelets via the translocation of the

- glucose transporter GLUT-3 from alpha-granules to the cell surface. *J Cell Biol*. 1997;138:323–330.
5. Sorbara LR, Davies-Hill TM, Koehler-Stec EM, Vannucci SJ, Horne MK, Simpson IA. Thrombin-induced translocation of GLUT3 glucose transporters in human platelets. *Biochem J*. 1997;328(pt 2):511–516.
 6. Chen LQ, Cheung LS, Feng L, Tanner W, Frommer WB. Transport of sugars. *Annu Rev Biochem*. 2015;84:865–894. doi: 10.1146/annurev-biochem-060614-033904.
 7. Deng D, Yan N. GLUT, SGLT, and SWEET: structural and mechanistic investigations of the glucose transporters. *Protein Sci*. 2016;25:546–558. doi: 10.1002/pro.2858.
 8. Craik JD, Stewart M, Cheeseman CI. GLUT-3 (brain-type) glucose transporter polypeptides in human blood platelets. *Thromb Res*. 1995;79:461–469.
 9. Fidler TP, Middleton EA, Rowley JW, Boudreau LH, Campbell RA, Souvenir R, Funari T, Tessander N, Boilard E, Weyrich AS, Abel ED. Glucose transporter 3 potentiates degranulation and is required for platelet activation. *Arterioscler Thromb Vasc Biol*. 2017;37:1628–1639. doi: 10.1161/ATVBAHA.117.309184.
 10. Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI. Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. *J Biol Chem*. 1988;263:15245–15248.
 11. Simpson IA, Dwyer D, Malide D, Moley KH, Travis A, Vannucci SJ. The facilitative glucose transporter GLUT3: 20 years of distinction. *Am J Physiol Endocrinol Metab*. 2008;295:E242–E253. doi: 10.1152/ajpendo.90388.2008.
 12. Maher F, Davies-Hill TM, Simpson IA. Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons. *Biochem J*. 1996;315(pt 3):827–831.
 13. Chicka MC, Ren Q, Richards D, Hellman LM, Zhang J, Fried MG, Whiteheart SW. Role of Munc13-4 as a Ca²⁺-dependent tether during platelet secretion. *Biochem J*. 2016;473:627–639. doi: 10.1042/BJ20151150.
 14. Koseoglu S, Dilks JR, Peters CG, Fitch-Tewfik JL, Fadel NA, Jasuja R, Italiano JE Jr, Haynes CL, Flaumenhaft R. Dynamin-related protein-1 controls fusion pore dynamics during platelet granule exocytosis. *Arterioscler Thromb Vasc Biol*. 2013;33:481–488. doi: 10.1161/ATVBAHA.112.255737.
 15. Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood*. 2012;120:e73–e82. doi: 10.1182/blood-2012-04-416594.
 16. Bordoli MR, Yum J, Breitkopf SB, Thon JN, Italiano JE Jr, Xiao J, Worby C, Wong SK, Lin G, Edenius M, Keller TL, Asara JM, Dixon JE, Yeo CY, Whitman M. A secreted tyrosine kinase acts in the extracellular environment. *Cell*. 2014;158:1033–1044. doi: 10.1016/j.cell.2014.06.048.
 17. Stalker TJ, Welsh JD, Tomaiuolo M, Wu J, Colace TV, Diamond SL, Brass LF. A systems approach to hemostasis: 3. thrombus consolidation regulates intrathrombus solute transport and local thrombin activity. *Blood*. 2014;124:1824–1831. doi: 10.1182/blood-2014-01-550319.

KEY WORDS: Editorials ■ glucose transporter type 3 ■ glycogenolysis ■ glycolysis ■ secretion