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Platelet Mitochondria: The Mighty Few

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Abstract

Purpose of review: Platelet mitochondrial dysfunction is both caused by, as well as a source of oxidative stress. Oxidative stress is a key hallmark of metabolic disorders such as dyslipidemia and diabetes which are known to have higher risks for thrombotic complications.

Recent findings: Increasing evidence supports a critical role for platelet mitochondria beyond energy production and apoptosis. Mitochondria are key regulators of reactive oxygen species and procoagulant platelets, which both contribute to pathological thrombosis. Studies targeting platelet mitochondrial pathways have reported promising results suggesting anti-thrombotic effects with limited impact on hemostasis in animal models.

Summary: Targeting platelet mitochondria holds promise for the reduction of thrombotic complications in patients with metabolic disorders. Future studies should aim at validating these preclinical findings and translate them to the clinic.

Keywords

Platelets; mitochondria; oxidative stress; diabetes; arterial thrombosis

Introduction

Textbooks commonly describe mitochondria as the powerhouse of the cell, and this holds true for platelets as well(1,2). This is due to the fact that mitochondria are responsible for important energy processes, including the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), which are crucial for the generation of adenosine triphosphate (ATP)(3,4). Mitochondria are double membrane organelles, composed of an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM)

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(5,6). These two membranes separate the mitochondria into two different spaces, the intermembrane space and the mitochondrial matrix(3,5). The OMM is composed of various porins allowing the diffusion of diverse molecules into the intermembrane space(5). The intermembrane space houses proteins that execute important functions in apoptosis and maintaining proper mitochondrial dynamics(7). Unlike the OMM, the IMM is highly impermeable thus, requiring various protein transporters for molecules to enter the matrix(4,5). More importantly, the IMM contains the electron transport chain (ETC) enzymes which are critical for energy production(6). The most inner compartment of the mitochondria is the matrix. The matrix contains all necessary components for the TCA, as well as molecules that regulate mitochondria antioxidative capacity(3,8).

Studies in the past 20 years have revealed a greater than expected complexity and versatility of mitochondrial activities(9). In addition to their primary role in ATP production, mitochondria are involved in other critical processes that contribute to platelet function and signaling, including reactive oxygen species (ROS) generation, Ca^{2+} homeostasis, and apoptosis(1,10). Platelets have relatively low mitochondrial counts (4-8 mitochondria per platelet), and are unable to replenish mitochondrial proteins encoded by nuclear DNA, because they lack a nucleus. This suggests that minimal alterations in platelet mitochondrial dynamics can have a significant impact on platelet function and hemostasis(1,11). An increasing number of studies are now investigating specific mitochondrial functions as potential therapeutic targets to prevent excessive platelet activation in cardiovascular disease(1,12). In this article, we will review the state of art regarding the role of platelet mitochondria in health and disease.

Fueling Platelets

Platelets are metabolically very active and need to maintain their high energy demand in order to preserve normal platelet function(13). Platelets respond to vascular tissue injury by clumping together (aggregating) and supporting the generation of fibrin to stabilize growing thrombi. Platelets generate energy by combining glycolysis and mitochondrial OXPHOS, with glycolysis being the predominant provider of cellular ATP (Figure 1)(14-16). To fully inhibit platelet function, mitochondrial OXPHOS and glycolysis need to be inhibited simultaneously(17). This is because platelets have a unique metabolic flexibility that allows them to utilize glycolysis or fatty acid catabolism instead of OXPHOS, or vice versa, depending on the situation and/or location they are in(18,19). This flexibility ensures platelets can function in a wide range of environments, where nutrients and oxygen may be limited, including within a growing thrombus.

While platelets operate at a relatively low metabolic rate while in the circulation, they can respond quickly to situations that require higher energy. Platelet glucose uptake increases in response to external stimuli and follows a distinct pattern during platelet activation(16,20). Platelet activation increases the glycolytic rate approximately two-fold compared to resting state (21,22). Importantly, platelets do this while preserving mitochondrial function regardless of fuel availability(18). Upon activation, platelets adopt an aerobic glycolytic phenotype which is less efficient than OXPHOS, but is faster, and necessary for platelets to quickly respond to external triggers(23). Glucose is abundant and readily available for

platelets; it is continuously taken up and is needed for normal function in both resting and activated platelets(24). Glucose enters platelets via the facilitative glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3). Murine studies have demonstrated that GLUT1 and GLUT3 exhibit overlapping, as well as unique functions(25,26). While GLUT1 is continuously present on the plasma membrane, GLUT3 is predominantly located on α-granule membranes with only ±15% localized in the plasma membrane(20,26). During platelet activation, GLUT3 translocates to the plasma membrane and mediates the increased glucose uptake following activation. Additionally, GLUT3 drives glucose uptake in α-granules which allows for intragranular glycolysis and is essential for proper degranulation(26). Interestingly, deletion of GLUT1 alone does not alter glucose uptake, indicating that GLUT3 may compensate for GLUT1(27). In contrast, deletion of GLUT3 only slightly decreased basal glucose uptake, but completely abolished agonist-mediated glucose uptake(26).

The preference for aerobic glycolysis in activated platelets is evident when cellular oxygen consumption is measured during platelet activation(28). Initially, post-activation, there is a short-lived but sharp rise in cellular oxygen consumption which rapidly declines before platelets reach full activation, and while they are still consuming ATP. This is a highly coordinated process, achieved by blocking pyruvate from oxidization in the TCA cycle, leading to a decline in mitochondrial respiration and an accompanying rise in lactic acid(2,28)(29). The metabolic checkpoint which determines whether glucose will be catabolized to lactate is the enzymatic activity of pyruvate kinase which generates pyruvate and the enzyme pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA(30). The activity of PDH is inhibited in activated platelets by pyruvate dehydrogenase kinase (PDK), which restricts flux of pyruvate into the TCA cycle to favor aerobic glycolysis(2,28). Pyruvate kinases catalyze the final step of glycolysis, which involves the irreversible transphosphorylation of phosphoenolpyruvate to produce pyruvate and ATP(31). While platelets express two isoforms of pyruvate kinase (PKM1 and PKM2), PKM2 expression was found to be higher compared to PKM1. Currently little is known on the role of PKM1 in platelets(2). Conversely, activation of platelets induces PKM2 dimerization, which is associated with enhanced catalytic activity, increasing glucose uptake and lactate production(32-34). Elegant inhibitor and platelet-specific knockout (KO) studies have reported on a critical role for the dimeric PKM2 isoform in regulating platelet integrin activation, aggregation, clot retraction, and thrombus formation under arterial shear in mice(2,28). In contrast to the potent anti-thrombotic effects of PKM2 inhibition, bleeding times were unaltered. Of note, ablation of platelet glucose uptake impaired both thrombosis and hemostasis, prolonging bleeding times in a similar model of hemostasis(27). Combined, these studies suggests that regulation of pyruvate rather than glucose, is an attractive target to develop a safe anti-thrombotic agent. However, more studies are needed towards this end.

Regulation of Mitochondrial Calcium

Mitochondrial calcium regulates ATP production, oxygen consumption and mitochondrial cell death by regulating various mitochondrial complexes, dehydrogenases and the formation of the mitochondrial permeability transition pore (mPTP)(8,28,35-37). In resting cells, mitochondrial Ca^{2+} levels are very similar to those in the cytoplasm. However, upon

activation, mitochondria can store up to 20-fold more Ca^{2+} (38). Mitochondria can take up these high amounts of Ca^{2+} because of their structure, which allows for several direct contact sites with Ca^{2+} stores and the presence of a highly selective Ca^{2+} channel located at the IMM(39-42).

Mitochondrial Ca^{2+} uptake is driven by a membrane potential difference, which is tightly linked to the ETC. Initially, Ca^{2+} is transported across the OMM through voltage-dependent anion selective channel proteins (VDACs). VDACs are highly specific for Ca^{2+} and allow Ca^{2+} transfer irrespective of their conformation. While, three VDAC isoforms are abundantly expressed in platelets (VDAC1, VDAC2 and VDAC3), little is known of their function(43). After passing through the OMM, Ca^{2+} crosses the IMM mainly through the mitochondrial calcium uniporter (MCU) complex found in the IMM (Figure 2). This transmembrane multimeric protein complex is composed of the pore-forming MCU subunit, the Ca^{2+} dependent regulating proteins mitochondrial calcium uptake 1 (MICU1) and MICU2, and the single pass membrane non-Ca^{2+} dependent regulatory protein essential MCU regulator (EMRE)(44,45). Dimerized MICU1 and MICU2 act as gatekeepers, limiting mitochondrial Ca^{2+} uptake in situations where cytosolic Ca^{2+} is significantly increased(46). Furthermore, Ca^{2+} uptake through MCU is negatively regulated by oligomerization of MCUb with MCU, decreasing mitochondrial Ca^{2+} flux(44,47,48). In platelets, MCU is a key regulator of procoagulant platelet formation(40). During robust platelet activation, high cytosolic Ca^{2+} will increase mitochondrial calcium uptake through MCU, ultimately leading to the opening of the mPTP(37,40). Furthermore, high mitochondrial Ca^{2+} flux can also lead to platelet apoptosis through the release of cytochrome C and the activation of caspases(49). Recently, a role for mitochondrial calcium was found in regulating platelet activation and arterial thrombosis. Platelets from MCUb global KO mice had altered cytoplasmic and mitochondrial Ca^{2+} flux, impaired ROS formation, reduced platelet aggregation, glucose uptake and bioenergetics(48). Combined, this resulted in an anti-thrombotic phenotype with delayed thrombus formation in several models of arterial thrombosis without prolonging tail-clip bleeding times(48). These results uncover a novel role for mitochondrial calcium that extend beyond procoagulant platelet formation or platelet apoptosis. However, the specific mechanisms of how mitochondrial calcium regulates platelet activation are yet to be described.

Critically, ablation of MCU does not completely abolish mitochondrial Ca^{2+} uptake in other cell types(38). Alternative Ca^{2+}uptake mechanisms that have been reported are the transient receptor potential channel 3 (TRPC3), mitochondrial uncoupling protein 2 (UCP2) and 3 (UCP3)(38). However, of these, only UCP2 is abundantly expressed in platelets(43).

Platelet Mitochondria Contribute to Thrombosis, Hemostasis and Beyond

As mitochondria fuel platelets, it is not unexpected that impairment of platelet mitochondrial functionality greatly impacts platelet thrombus formation. However, platelet mitochondria themselves also impact thrombus formation, independent of their energetic function. They accomplish this through procoagulant platelet formation and mitochondrial ROS formation (Figure 2)(37,50).

Procoagulant platelet formation, often referred to as platelet necrosis, occurs when cytosolic Ca^{2+} levels remain elevated due to strong platelet activation(51). These sustained high cytosolic Ca^{2+} levels result in mitochondria storing increasing amounts of Ca^{2+}(52,53). Whenever a critical mitochondrial calcium threshold is reached, the mitochondrial membranes depolarize, initiating the opening of the mPTP(36,54). The opening of the mPTP further increases cytosolic Ca^{2+}, activating the scramblase TMEM16F(55). At the same time, mitochondria will depolarize, depleting ATP and inhibiting flippases(49). These processes ultimately result in translocation of negatively charged phosphatidylserine (PS) from the inner to the outer membrane of the platelet lipid bilayer. This allows coagulation factors to bind to the negatively charged PS on the platelet surface and initiate thrombin generation(55).

Similarly, during platelet activation, mitochondria will generate ROS as a byproduct from increased ATP production(56). Mitochondrial redox homeostasis is regulated by antioxidant enzymes, of which superoxide dismutase 2 (SOD2) is the most important in platelets(57). Mitochondrial ROS can further activate platelet signaling, creating an activation amplification loop which contributes to thrombotic complications in diabetes and sickle cell disease(58-60). While not yet fully elucidated, ROS have been found to alter platelet receptor activity, platelet secretion events, bioavailability of platelet agonists and inhibitors, and ROS have also been reported to directly induce platelet signaling(61-66). Whether this is specifically dependent on cytoplasmic or mitochondrial ROS remains unclear. Lastly, mitochondrial ROS levels are also regulated by the mitochondrial membrane potential and therefore involved in procoagulant platelet formation(52). Importantly, the prothrombotic effects of mitochondrial ROS are more pronounced in aged individuals, where thrombotic risk is elevated(67).

Another remarkable feature of activated platelets is their ability to release their mitochondria as part of extracellular vesicles(68). Extracellular mitochondria are potent triggers for neutrophil migration and neutrophil extracellular trap formation, and have been associated with adverse transfusion reactions(68-71). Likewise, extracellular platelet mitochondria were found inside neutrophils isolated from the joints of arthritic patients(72). Finally, in systemic lupus erythematosus patients, extracellular mitochondrial DNA originating from platelets was found to be a potent source for autoantibodies. In contrast to these findings, free platelet mitochondria also have been reported to improve the regenerative capacity of mesenchymal stem cells by stimulating fatty acid synthesis(73,74).

Pathologic Mitochondrial Function in Platelets

Co-morbidities such as diabetes and obesity greatly impact thrombotic risk, and this is partially attributed to their impact on platelet function. In particular, oxidative stress is known to contribute to diabetes and obesity by exposing platelets to a prothrombotic environment and by lowering the threshold to form the mPTP and depolarize mitochondria(75,76). Hyperpolarization of the mitochondrial membrane will increase mitochondrial ROS production, amplifying oxidative stress(58,77).

Diabetes Mellitus is an independent risk factor for atherosclerosis and thrombosis after vascular interventions. Platelet hyperreactivity is commonly associated with diabetes and is a key contributing factor to the prothrombotic conditions observed in diabetic patients(78). Specific mechanisms leading to increased platelet reactivity have not fully been elucidated, but include increased platelet receptor density, increased Ca^{2+} signaling and hyperreactivity to platelet agonists(79-81). Recent work linked platelet mitochondrial impairment to the dysregulation in platelet function observed in diabetes(82,83). Mitochondrial stress tests, which calculate mitochondrial efficiency in various metabolic processes, have shown that resting platelets from diabetic patients have lower maximal respiration compared to non-diabetic patients(82-84). Activation of diabetic platelets with thrombin further accentuated differences in maximal respiration, while also demonstrating a decrease in basal oxygen rate consumption (OCR) and ATP production(82). Furthermore, these deficiencies in mitochondrial function were further exacerbated in diabetic patients with thrombotic complications(82,85). In addition to alterations in mitochondrial respiration, platelets from diabetic patients with thrombotic complications showed further alterations in mitochondrial complex I, and had an increase in mitochondrial ROS, P-selectin surface expression and aggregation(82). Interestingly, this phenotype could be reversed by inhibiting complex I with metformin(82,83).

Most of the effects of diabetes on platelet function have been attributed to hyperglycemia, suggesting control of blood glucose levels is critical for the prevention of thrombotic complications in diabetic patients(86). Increasing glucose excretion and lowering blood glucose levels with sodium glucose cotransporter-2 inhibitors for example, greatly improves cardiovascular outcomes in patients with diabetes(87,88). *In vitro* and murine studies have found hyperglycemia, independently from diabetes, increases platelet activation, platelet clearance and contributes to *in vivo* thrombosis(76). Moreover, hyperglycemic mice are more potent at generating procoagulant platelets and produce more mitochondrial ROS(89). These observations are congruent with the altered mitochondrial respiration and dynamics observed in mice with diabetes. Importantly, deletion of the main platelet glucose transporters GLUT 1 and 3 reversed this phenotype, normalizing platelet respiration, activation and platelet induced arterial and pulmonary thrombosis(76). Conversely, in the setting of ischemic stroke, alteration in platelet glucose metabolism did not reduce hyperglycemia induced exacerbation of stroke(89). These results imply a more complex role for hyperglycemia in ischemic stroke.

Mechanistically, hyperglycemia induces aldose reductase activation(90), resulting in ROS production to induce phosphorylation of p53. Phosphorylated p53 then induces a loss of mitochondrial membrane potential(91). Platelets attempt to compensate for this loss in mitochondrial membrane potential by upregulating mitophagy, as increased mitophagy reduced mitochondrial stress and diabetes associated thrombosis in mice(92). Additionally, several studies hint to the possibility of mitochondrial calcium playing an important role in platelet activation in diabetes. Indeed, studies have demonstrated complex I regulates the turnover of MCU, increasing mitochondrial Ca^{2+} levels(93,94).

Dyslipidemia is characterized by abnormal levels of cholesterol, triglycerides, and dietary fatty acids. There is strong evidence supporting an association between dyslipidemia and

a lower threshold for platelet activation, particularly in patients with heart attack and stroke(95,96). Furthermore, recent evidence also points towards an association between dyslipidemia and the risk of venous thrombotic events(97,98). In dyslipidemia, platelets are sensitized to activation by oxidized phospholipids found in low-density lipoprotein particles (oxLDL), which are abundantly found in plasma of coronary artery disease patients(58,99). OxLDL activates platelets by interacting with the platelet receptor CD36(100). CD36 induces platelet activation through amplification of glycoprotein VI (GPVI) downstream signaling, ROS production through NADPH oxidase, and by desensitizing the inhibitory cGMP / platelet protein kinase G pathway(101,102).

Mechanistically, CD36 generates ROS through NADPH oxidase activates ERK5 and induces platelet aggregation and coagulation, through PS exposure on the platelet surface(103). The latter is distinct from PS exposure mediated by the formation of the mPTP (procoagulant platelet) or platelet apoptosis(104). CD36- and ERK5-mediated PS exposure requires Src family kinases, hydrogen peroxide, and apoptotic caspases(103). Importantly, CD36-ERK5-caspase signaling needs to cooperate with the GPVI pathway for the externalization of PS, as oxLDL alone was not sufficient in supporting thrombin generation and fibrin formation. This implies a unique way for platelets to expose PS under metabolic stress that combines both features of procoagulant platelets and apoptosis(105).

Besides CD36, other scavenging receptors involved in dyslipidemia associated thrombosis include Lectin-like Oxidized Low-Density Receptor 1 (LOX1), Scavenger Receptor A1 (SR-A1) and several toll-like receptors (TLRs) (106). However, the signaling effectors associated with these receptors do not involve mitochondrial platelet dysfunction.

Conclusion

Increasing evidence supports a critical role for platelet mitochondria beyond energy production and apoptosis. Generation of ROS and the formation of procoagulant platelets contribute to pathological thrombosis and are increased in metabolic disorders. Preclinical studies targeting platelet mitochondrial pathways have yielded promising thrombo-protective results with limited impact on hemostasis. Future studies should aim at validating these findings and translate them to the clinic.

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Reference List

1. Melchinger H, Jain K, Tyagi T, Hwa J. Role of Platelet Mitochondria: Life in a Nucleus-Free Zone. Front Cardiovasc Med. 2019 Oct 29;6:153. [PubMed: 31737646]

2. Nayak MK, Dhanesha N, Doddapattar P, et al. Dichloroacetate, an inhibitor of pyruvate dehydrogenase kinases, inhibits platelet aggregation and arterial thrombosis. Blood Adv. 2018 Aug 14;2(15):2029–38. [PubMed: 30108111]

3. Glancy B, Kim Y, Katti P, Willingham TB. The Functional Impact of Mitochondrial Structure Across Subcellular Scales. Front Physiol. 2020 Nov 11;11:541040. [PubMed: 33262702]

4. Friedman JR, Nunnari J. Mitochondrial form and function. Nature. 2014 Jan 16;505(7483):335–43. [PubMed: 24429632]

5. McCarron JG, Wilson C, Sandison ME, et al. From Structure to Function: Mitochondrial Morphology, Motion and Shaping in Vascular Smooth Muscle. J Vasc Res. 2013;50(5):357–71. [PubMed: 23887139]

6. Kühlbrandt W. Structure and function of mitochondrial membrane protein complexes. BMC Biol. 2015 Dec;13(1):89. [PubMed: 26515107]

7. Edwards R, Gerlich S, Tokatlidis K. The biogenesis of mitochondrial intermembrane space proteins. Biological Chemistry. 2020 May 26;401(6–7):737–47. [PubMed: 32061164]

8. Glancy B, Balaban RS. Role of Mitochondrial Ca^{2+} in the Regulation of Cellular Energetics. Biochemistry. 2012 Apr 10;51(14):2959–73. [PubMed: 22443365]

9. Pfanner N, Warscheid B, Wiedemann N. Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol. 2019 May;20(5):267–84. [PubMed: 30626975]

10. Bertero E, Maack C. Calcium Signaling and Reactive Oxygen Species in Mitochondria. Circ Res. 2018 May 11;122(10):1460–78. [PubMed: 29748369]

11. Baccarelli AA, Byun HM. Platelet mitochondrial DNA methylation: a potential new marker of cardiovascular disease. Clin Epigenet. 2015 Dec;7(1):44.

12. Blagov AV, Kozlov S, Blokhina T, Sukhorukov VN, Orekhov AN. Targeting Mitochondrial Dynamics Proteins for the Development of Therapies for Cardiovascular Diseases. Int J Mol Sci. 2022 Nov 25;23(23):14741. [PubMed: 36499064]

13. Kramer PA, Ravi S, Chacko B, et al. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol. 2014;2:206–10. [PubMed: 24494194]

14. Chacko BK, Kramer PA, Ravi S, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Lab Invest. 2013 Jun;93(6):690–700. [PubMed: 23528848]

15. Doery JC, Hirsh J, Cooper I. Energy metabolism in human platelets: interrelationship between glycolysis and oxidative metabolism. Blood. 1970 Aug;36(2):159–68. [PubMed: 5427451]

16. 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 May;57(5):956–66. [PubMed: 6260270]

17. Holmsen H, Setkowsky CA, Day HJ. Effects of antimycin and 2-deoxyglucose on adenine nucleotides in human platelets. Role of metabolic adenosine triphosphate in primary aggregation, secondary aggregation and shape change of platetets. Biochem J. 1974 Nov;144(2):385–96. [PubMed: 4462589]

18. Aibibula M, Naseem KM, Sturmey RG. Glucose metabolism and metabolic flexibility in blood platelets. J Thromb Haemost. 2018 Nov;16(11):2300–14. [PubMed: 30151891]

19. Ravi S, Chacko B, Sawada H, et al. Metabolic plasticity in resting and thrombin activated platelets. PLoS One. 2015;10(4):e0123597. [PubMed: 25875958]

20. Heijnen HF, Oorschot V, Sixma JJ, et al. 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 Jul 28;138(2):323–30. [PubMed: 9230074]

21. Warshaw AL, Laster L, Shulman NR. The stimulation by thrombin of glucose oxidation in human platelets. J Clin Invest. 1966 Dec;45(12):1923–34. [PubMed: 4959090]

22. Karpatkin S Studies on human platelet glycolysis. Effect of glucose, cyanide, insulin, citrate, and agglutination and contraction on platelet glycolysis. J Clin Invest. 1967 Mar;46(3):409–17. [PubMed: 6023775]

23. Pfeiffer T, Schuster S, Bonhoeffer S. Cooperation and competition in the evolution of ATP-producing pathways. Science. 2001 Apr 20;292(5516):504–7. [PubMed: 11283355]

24. Akkerman JW, Holmsen H, Driver HA. Platelet aggregation and Ca2+ secretion are independent of simultaneous ATP production. FEBS Lett. 1979 Apr 15;100(2):286–90. [PubMed: 37114]
25. Gauer JS, Ajjan RA, Ariëns RAS. Platelet–Neutrophil Interaction and Thromboinflammation in Diabetes: Considerations for Novel Therapeutic Approaches. JAHA. 2022 Oct 18;11(20):e027071. [PubMed: 36250653]
26. Fidler TP, Middleton EA, Rowley JW, et al. Glucose Transporter 3 Potentiates Degranulation and Is Required for Platelet Activation. ATVB. 2017 Sep;37(9):1628–39.
27. Fidler TP, Campbell RA, Funari T, et al. Deletion of GLUT1 and GLUT3 Reveals Multiple Roles for Glucose Metabolism in Platelet and Megakaryocyte Function. Cell Rep. 2017 Jul 25;20(4):881–94. [PubMed: 28746873]
**28. Nayak MK, Ghatge M, Flora GD, et al. The metabolic enzyme pyruvate kinase M2 regulates platelet function and arterial thrombosis. Blood. 2021 Mar 25;137(12):1658–68. [PubMed: 33027814] This study elegantly demonstrated the therapeutic potential of targeting platlet PKM2 as a safe antithrombotic strategy.
29. Kobzar G, Mardla V, Samel N. Lactate is a possible mediator of the glucose effect on platelet inhibition. Platelets. 2014;25(4):239–45. [PubMed: 23909711]
30. Gray LR, Tompkins SC, Taylor EB. Regulation of pyruvate metabolism and human disease. Cell Mol Life Sci. 2014 Jul;71(14):2577–604. [PubMed: 24363178]
31. Zhang S, Hulver MW, McMillan RP, et al. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr Metab (Lond). 2014 Feb 12;11(1):10. [PubMed: 24520982]
32. Holness MJ, Sugden MC. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem Soc Trans. 2003 Dec;31(Pt 6):1143–51. [PubMed: 14641014]
33. Wu CA, Chao Y, Shiah SG, Lin WW. Nutrient deprivation induces the Warburg effect through ROS/AMPK-dependent activation of pyruvate dehydrogenase kinase. Biochim Biophys Acta. 2013 May;1833(5):1147–56. [PubMed: 23376776]
34. Hitosugi T, Kang S, Vander Heiden MG, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal. 2009 Nov 17;2(97):ra73. [PubMed: 19920251]
35. Kulkarni PP, Tiwari A, Singh N, et al. Aerobic glycolysis fuels platelet activation: small-molecule modulators of platelet metabolism as anti-thrombotic agents. Haematologica. 2019 Apr;104(4):806–18. [PubMed: 30381300]
36. Jobe SM, Wilson KM, Leo L, et al. Critical role for the mitochondrial permeability transition pore and cyclophilin D in platelet activation and thrombosis. Blood. 2008 Feb 1;111(3):1257–65. [PubMed: 17989312]
37. Denorme F, Campbell RA. Procoagulant platelets: novel players in thromboinflammation. Am J Physiol Cell Physiol. 2022 Oct 1;323(4):C951–8. [PubMed: 35993516]
38. Giorgi C, Marchi S, Pinton P. The machineries, regulation and cellular functions of mitochondrial calcium. Nat Rev Mol Cell Biol. 2018 Nov;19(11):713–30. [PubMed: 30143745]
*39. Wu B, Ye Y, Xie S, et al. Megakaryocytes Mediate Hyperglycemia-Induced Tumor Metastasis. Cancer Research. 2021 Nov 1;81(21):5506–22. [PubMed: 34535458] Identified a role for GRP75 in hyperglycemic platelet activation through regulating ER-mitochondrial Ca2+ transfer.
40. Kholmukhamedov A, Janecke R, Choo HJ, Jobe SM. The mitochondrial calcium uniporter regulates procoagulant platelet formation. J Thromb Haemost. 2018 Nov;16(11):2315–21. [PubMed: 30179298]
41. Chaudhuri D, Sancak Y, Mootha VK, Clapham DE. MCU encodes the pore conducting mitochondrial calcium currents. eLife. 2013 Jun 4;2:e00704. [PubMed: 23755363]
42. Panahi G, Pasalar P, Zare M, et al. MCU-knockdown attenuates high glucose-induced inflammation through regulating MAPKs/NF-κB pathways and ROS production in HepG2 cells. Kanzaki M, editor. PLoS ONE. 2018 Apr 30;13(4):e0196580. [PubMed: 29709004]
43. Simon LM, Edelstein LC, Nagalla S, et al. Human platelet microRNA-mRNA networks associated with age and gender revealed by integrated plateletomics. Blood. 2014 Apr 17;123(16):e37–45. [PubMed: 24523238]
44. Marchi S, Pinton P. The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications: The mitochondrial calcium uniporter complex. The Journal of Physiology. 2014 Mar 1;592(5):829–39. [PubMed: 24366263]

45. Alevriadou BR, Patel A, Noble M, et al. Molecular nature and physiological role of the mitochondrial calcium uniporter channel. American Journal of Physiology-Cell Physiology. 2021 Apr 1;320(4):C465–82. [PubMed: 33296287]

46. Mallilankaraman K, Doonan P, Cárdenas C, et al. MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca(2+) uptake that regulates cell survival. Cell. 2012 Oct 26;151(3):630–44. [PubMed: 23101630]

47. Lambert JP, Luongo TS, Tomar D, et al. MCUB Regulates the Molecular Composition of the Mitochondrial Calcium Uniporter Channel to Limit Mitochondrial Calcium Overload During Stress. Circulation. 2019 Nov 19;140(21):1720–33. [PubMed: 31533452]

*48. Ghatge M, Nayak MK, Flora GD, et al. Mitochondrial calcium uniporter b deletion inhibits platelet function and reduces susceptibility to arterial thrombosis. Journal of Thrombosis and Haemostasis. 2023 Apr;S1538783623003161. Demonstrated mitochondrial calcium as a regulator of platelet activation.

49. Millington-Burgess SL, Harper MT. Cytosolic and mitochondrial Ca $^{2+}$ signaling in procoagulant platelets. Platelets. 2021 Oct 3;32(7):855–62. [PubMed: 33600275]

50. Masselli E, Pozzi G, Vaccarezza M, et al. ROS in Platelet Biology: Functional Aspects and Methodological Insights. Int J Mol Sci. 2020 Jul 9;21(14):4866. [PubMed: 32660144]

51. Mammadova-Bach E, Nagy M, Heemskerk JWM, et al. Store-operated calcium entry in thrombosis and thrombo-inflammation. Cell Calcium. 2019 Jan;77:39–48. [PubMed: 30530092]

52. Choo HJ, Saafir TB, Mkumba L, et al. Mitochondrial Calcium and Reactive Oxygen Species Regulate Agonist-Initiated Platelet Phosphatidylserine Exposure. ATVB. 2012 Dec;32(12):2946–55.

53. Abbasian N, Millington-Burgess SL, Chabra S, et al. Supramaximal calcium signaling triggers procoagulant platelet formation. Blood Advances. 2020 Jan 14;4(1):154–64. [PubMed: 31935287]

54. Remenyi G, Szasz R, Friese P, Dale GL. Role of Mitochondrial Permeability Transition Pore in Coated-Platelet Formation. ATVB. 2005 Feb;25(2):467–71.

55. van Kruchten R, Mattheij NJA, Saunders C, et al. Both TMEM16F-dependent and TMEM16F-independent pathways contribute to phosphatidylserine exposure in platelet apoptosis and platelet activation. Blood. 2013 Mar 7;121(10):1850–7. [PubMed: 23303820]

56. Zharikov S, Shiva S. Platelet mitochondrial function: from regulation of thrombosis to biomarker of disease. Biochem Soc Trans. 2013 Feb 1;41(1):118–23. [PubMed: 23356269]

57. Fidler TP, Rowley JW, Araujo C, et al. Superoxide Dismutase 2 is dispensable for platelet function. Thromb Haemost. 2017;117(10):1859–67. [PubMed: 28771279]

58. Yamagishi S ichi, Edelstein D, Du X liang, Brownlee M. Hyperglycemia Potentiates Collagen-Induced Platelet Activation Through Mitochondrial Superoxide Overproduction. Diabetes. 2001 Jun;50(6):1491–4. [PubMed: 11375352]

59. Cardenes N, Corey C, Geary L, et al. Platelet bioenergetic screen in sickle cell patients reveals mitochondrial complex V inhibition, which contributes to platelet activation. Blood. 2014 May 1;123(18):2864–72. [PubMed: 24677541]

*60. Annarapu GK, Nolfi-Donegan D, Reynolds M, et al. Mitochondrial reactive oxygen species scavenging attenuates thrombus formation in a murine model of sickle cell disease. J Thromb Haemost. 2021 Sep;19(9):2256–62. [PubMed: 33724688] Specific targeting of mitochondrial ROS reduced thrombotic burden in a model of sickle cell disease.

61. Kim K, Li J, Tseng A, Andrews RK, Cho J. NOX2 is critical for heterotypic neutrophil-platelet interactions during vascular inflammation. Blood. 2015 Oct 15;126(16):1952–64. [PubMed: 26333777]

62. Hosseini E, Solouki A, Roudsari ZO, K, et al. Reducing state attenuates ectodomain shedding of GPVI while restoring adhesion capacities of stored platelets: evidence addressing the controversy around the effects of redox condition on thrombosis. J Thromb Thrombolysis. 2020 Jul;50(1):123–34. [PubMed: 32409937]

63. Bakdash N, Williams MS. Spatially distinct production of reactive oxygen species regulates platelet activation. Free Radic Biol Med. 2008 Jul 15;45(2):158–66. [PubMed: 18452718]

64. Krötz F, Sohn HY, Pohl U. Reactive Oxygen Species: Players in the Platelet Game. ATVB. 2004 Nov;24(11):1988–96.

65. Dangel O, Mergia E, Karlisch K, et al. Nitric oxide-sensitive guanylyl cyclase is the only nitric oxide receptor mediating platelet inhibition. J Thromb Haemost. 2010 Jun;8(6):1343–52. [PubMed: 20149081]

66. Qiao J, Arthur JF, Gardiner EE, et al. Regulation of platelet activation and thrombus formation by reactive oxygen species. Redox Biol. 2018 Apr;14:126–30. [PubMed: 28888895]

*67. Sonkar VK, Eustes AS, Ahmed A, et al. Endogenous SOD2 (Superoxide Dismutase) Regulates Platelet-Dependent Thrombin Generation and Thrombosis During Aging. Arterioscler Thromb Vasc Biol. 2023 Jan;43(1):79–91. [PubMed: 36325902] SOD2 as a regulator of mitochondrial stress in platelets from aged mice.

68. Boudreau LH, Duchez AC, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. Blood. 2014 Oct 2;124(14):2173–83. [PubMed: 25082876]

69. Duchez AC, Boudreau LH, Naika GS, et al. Platelet microparticles are internalized in neutrophils via the concerted activity of 12-lipoxygenase and secreted phospholipase A2-IIA. Proc Natl Acad Sci U S A. 2015 Jul 7;112(27):E3564–3573. [PubMed: 26106157]

70. Su M, Chen C, Li S, et al. Gasdermin D-dependent platelet pyroptosis exacerbates NET formation and inflammation in severe sepsis. Nat Cardiovasc Res. 2022;1(8):732–47. [PubMed: 35967457]

71. Marcoux G, Magron A, Sut C, et al. Platelet-derived extracellular vesicles convey mitochondrial DAMPs in platelet concentrates and their levels are associated with adverse reactions. Transfusion. 2019 Jul;59(7):2403–14. [PubMed: 30973972]

72. Correction for Duchez et al., Platelet microparticles are internalized in neutrophils via the concerted activity of 12-lipoxygenase and secreted phospholipase A2-IIA. Proc Natl Acad Sci U S A. 2015 Dec 8;112(49):E6825. [PubMed: 26621721]

**73. Melki I, Allaeys I, Tessandier N, et al. Platelets release mitochondrial antigens in systemic lupus erythematosus. Sci Transl Med. 2021 Feb 17;13(581):eaav5928. [PubMed: 33597264] Platelets release mitochondria which mediate disease progression in systemic lupus erythematosus.

**74. Levoux J, Prola A, Lafuste P, et al. Platelets Facilitate the Wound-Healing Capability of Mesenchymal Stem Cells by Mitochondrial Transfer and Metabolic Reprogramming. Cell Metab. 2021 Feb 2;33(2):283–299.e9. [PubMed: 33400911] Platelet-released mitochondria induce reprogramming of stem cells.

75. Yan S, Du F, Wu L, et al. F1F0 ATP Synthase-Cyclophilin D Interaction Contributes to Diabetes-Induced Synaptic Dysfunction and Cognitive Decline. Diabetes. 2016 Nov;65(11):3482–94. [PubMed: 27554467]

76. Fidler TP, Marti A, Gerth K, et al. Glucose Metabolism Is Required for Platelet Hyperactivation in a Murine Model of Type 1 Diabetes. Diabetes. 2019 May;68(5):932–8. [PubMed: 30765335]

77. Sambola A, Ruiz-Meana M, Barba I, et al. Glycative and oxidative stress are associated with altered thrombus composition in diabetic patients with ST-elevation myocardial infarction. Int J Cardiol. 2017 Sep 15;243:9–14. [PubMed: 28747040]

78. Rodriguez BAT, Johnson AD. Platelet Measurements and Type 2 Diabetes: Investigations in Two Population-Based Cohorts. Front Cardiovasc Med. 2020 Jul 10;7:118. [PubMed: 32754618]

79. Watala C, Boncler M, Golański J, et al. Platelet membrane lipid fluidity and intraplatelet calcium mobilization in type 2 diabetes mellitus. Eur J Haematol. 1998 Nov;61(5):319–26. [PubMed: 9855247]

80. Soma P, Swanepoel AC, du Plooy JN, et al. Flow cytometric analysis of platelets type 2 diabetes mellitus reveals “angry” platelets. Cardiovasc Diabetol. 2016 Mar 31;15:52. [PubMed: 27036108]

81. Fejes Z, Póliska S, Czimmerer Z, et al. Hyperglycaemia suppresses microRNA expression in platelets to increase P2RY12 and SELP levels in type 2 diabetes mellitus. Thromb Haemost. 2017 Feb 28;117(3):529–42. [PubMed: 27975100]

**82. Gao MJ, Cui NH, Liu X, Wang XB. Inhibition of mitochondrial complex I leading to NAD+/NADH imbalance in type 2 diabetic patients who developed late stent thrombosis: Evidence from an integrative analysis of platelet bioenergetics and metabolomics. Redox Biol. 2022 Oct 11;57:102507. [PubMed: 36244294] Found that targeting mitochondrial complex I can regulate platelet activation in patients with type 2 diabetes that have suffered thrombotic complications.

83. Siewiera K, Labieniec-Watala M, Kassassir H, et al. Potential Role of Mitochondria as Modulators of Blood Platelet Activation and Reactivity in Diabetes and Effect of Metformin on Blood Platelet Bioenergetics and Platelet Activation. IJMS. 2022 Mar 27;23(7):3666. [PubMed: 35409027]

84. Gauer JS, Ajjan RA, Ariëns RAS. Platelet-Neutrophil Interaction and Thromboinflammation in Diabetes: Considerations for Novel Therapeutic Approaches. J Am Heart Assoc. 2022 Oct 18;11(20):e027071. [PubMed: 36250653]

*85. Lin ML, Fu TC, Hsu CC, et al. Cycling Exercise Training Enhances Platelet Mitochondrial Bioenergetics in Patients with Peripheral Arterial Disease: A Randomized Controlled Trial. Thromb Haemost. 2021 Jul;121(07):900–12. [PubMed: 33421964] Cycling improves platelet mitochondrial function in patients with peripheral arterial disease.

86. Fitchett D, Inzucchi SE, Cannon CP, et al. Empagliflozin Reduced Mortality and Hospitalization for Heart Failure Across the Spectrum of Cardiovascular Risk in the EMPA-REG OUTCOME Trial. Circulation. 2019 Mar 12;139(11):1384–95. [PubMed: 30586757]

87. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2019 Jan 24;380(4):347–57. [PubMed: 30415602]

*88. Chang SN, Chen JJ, Huang PS, et al. Sodium-Glucose Cotransporter-2 Inhibitor Prevents Stroke in Patients With Diabetes and Atrial Fibrillation. J Am Heart Assoc. 2023 May 16;12(10):e027764. [PubMed: 37183872] Demonstrated the efficacy of blocking glucose uptake to reducing thrombotic events in diabetic patients.

89. Denorme F, Portier I, Kosaka Y, Campbell RA. Hyperglycemia exacerbates ischemic stroke outcome independent of platelet glucose uptake. J Thromb Haemost. 2021 Feb;19(2):536–46. [PubMed: 33118271]

90. Tang WH, Stitham J, Gleim S, et al. Glucose and collagen regulate human platelet activity through aldose reductase induction of thromboxane. J Clin Invest. 2011 Nov;121(11):4462–76. [PubMed: 22005299]

91. Tang WH, Stitham J, Jin Y, et al. Aldose reductase-mediated phosphorylation of p53 leads to mitochondrial dysfunction and damage in diabetic platelets. Circulation. 2014 Apr 15;129(15):1598–609. [PubMed: 24474649]

92. Lee SH, Du J, Stitham J, et al. Inducing mitophagy in diabetic platelets protects against severe oxidative stress. EMBO Mol Med. 2016 Jul;8(7):779–95. [PubMed: 27221050]

**93. Balderas E, Eberhardt DR, Lee S, et al. Mitochondrial calcium uniporter stabilization preserves energetic homeostasis during Complex I impairment. Nat Commun. 2022 May 19;13(1):2769. [PubMed: 35589699] Increases in mitochondrial calcium through the MCU is critical in the proper function of cells with impaired complex I.

94. Marcu R, Wiczer BM, Neeley CK, Hawkins BJ. Mitochondrial Matrix Ca^{2+} Accumulation Regulates Cytosolic NAD^{+}/NADH Metabolism, Protein Acetylation, and Sirtuin Expression. Molecular and Cellular Biology. 2014 Aug 1;34(15):2890–902. [PubMed: 24865966]

95. Betteridge DJ, Cooper MB, Saggerson ED, et al. Platelet function in patients with hypercholesterolaemia. Eur J Clin Invest. 1994 Feb;24 Suppl 1:30–3. [PubMed: 8013529]

96. Lacoste L, Lam JY, Hung J, et al. Hyperlipidemia and coronary disease. Correction of the increased thrombogenic potential with cholesterol reduction. Circulation. 1995 Dec 1;92(11):3172–7. [PubMed: 7586300]

97. Stewart LK, Kline JA. Metabolic Syndrome Increases Risk of Venous Thromboembolism Recurrence after Acute Pulmonary Embolism. Ann Am Thorac Soc. 2020 Jul;17(7):821–8. [PubMed: 32187500]

98. Stewart LK, Kline JA. Metabolic syndrome increases risk of venous thromboembolism recurrence after acute deep vein thrombosis. Blood Adv. 2020 Jan 14;4(1):127–35. [PubMed: 31917844]

99. Stellos K, Sauter R, Fahrleitner M, et al. Binding of oxidized low-density lipoprotein on circulating platelets is increased in patients with acute coronary syndromes and induces platelet adhesion to vascular wall in vivo--brief report. Arterioscler Thromb Vasc Biol. 2012 Aug;32(8):2017–20. [PubMed: 22701021]

100. Podrez EA, Byzova TV, Febbraio M, et al. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. Nat Med. 2007 Sep;13(9):1086–95. [PubMed: 17721545]

101. Magwenzi S, Woodward C, Wraith KS, et al. Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/protein kinase G signaling cascade. Blood. 2015 Apr 23;125(17):2693–703. [PubMed: 25710879]

102. Berger M, Raslan Z, Aburima A, et al. Atherogenic lipid stress induces platelet hyperactivity through CD36-mediated hyposensitivity to prostacyclin: the role of phosphodiesterase 3A. Haematologica. 2020 Mar;105(3):808–19. [PubMed: 31289200]

103. Yang M, Kholmukhamedov A, Schulte ML, et al. Platelet CD36 signaling through ERK5 promotes caspase-dependent procoagulant activity and fibrin deposition in vivo. Blood Adv. 2018 Nov 13;2(21):2848–61. [PubMed: 30381401]

104. Schoenwaelder SM, Yuan Y, Josefsson EC, et al. Two distinct pathways regulate platelet phosphatidylserine exposure and procoagulant function. Blood. 2009 Jul 16;114(3):663–6. [PubMed: 19387006]

105. Yang M, Silverstein RL. CD36 and ERK5 link dyslipidemia to apoptotic-like platelet procoagulant function. Curr Opin Hematol. 2019 Sep;26(5):357–65. [PubMed: 31261174]

106. Berger M, Naseem KM. Oxidised Low-Density Lipoprotein-Induced Platelet Hyperactivity-Receptors and Signalling Mechanisms. Int J Mol Sci. 2022 Aug 16;23(16):9199. [PubMed: 36012465]

Key Points

- Platelets are metabolically flexible and switch energy source depending on the situation they are in.
- Regulation of pyruvate, rather than glucose, is an attractive safe anti-thrombotic target.
- Platelet mitochondria contribute to thrombosis through the formation of procoagulant platelets and mitochondrial ROS.
- Mitochondrial stress is increased in diabetes and dyslipidemia and contributes to increased thrombosis.

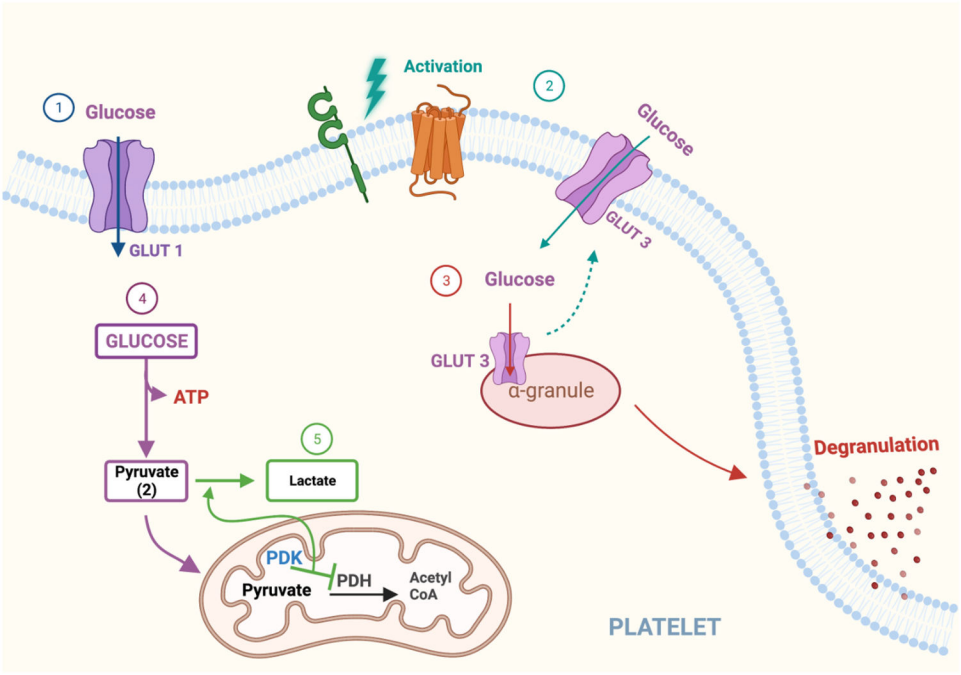

Figure 1. Fueling platelets with glucose is mediated by GLUT1 and GLUT3.

(1) Under basal conditions, GLUT1 (Glucose transporter 1) is the primary glucose transporter on platelets. (2) Upon platelet activation, GLUT3 translocates to the plasma membrane and mediates increased glucose uptake. (3) Additionally, GLUT3 drives glucose uptake in α-granules which allows for proper degranulation. (4) Platelets generate energy by combining glycolysis and mitochondrial OXPHOS (oxidative phosphorylation), with aerobic glycolysis being the predominant provider of cellular ATP. (5) Platelets achieve this by inhibiting the oxidation of pyruvate, which is mediated by PDK (pyruvate dehydrogenase kinase) blocking PDH (pyruvate dehydrogenase)-mediated catabolization of pyruvate. This blocks pyruvate from entering the TCA (tricarboxylic acid cycle) cycle preventing a decline in mitochondrial respiration and increases production of lactate.

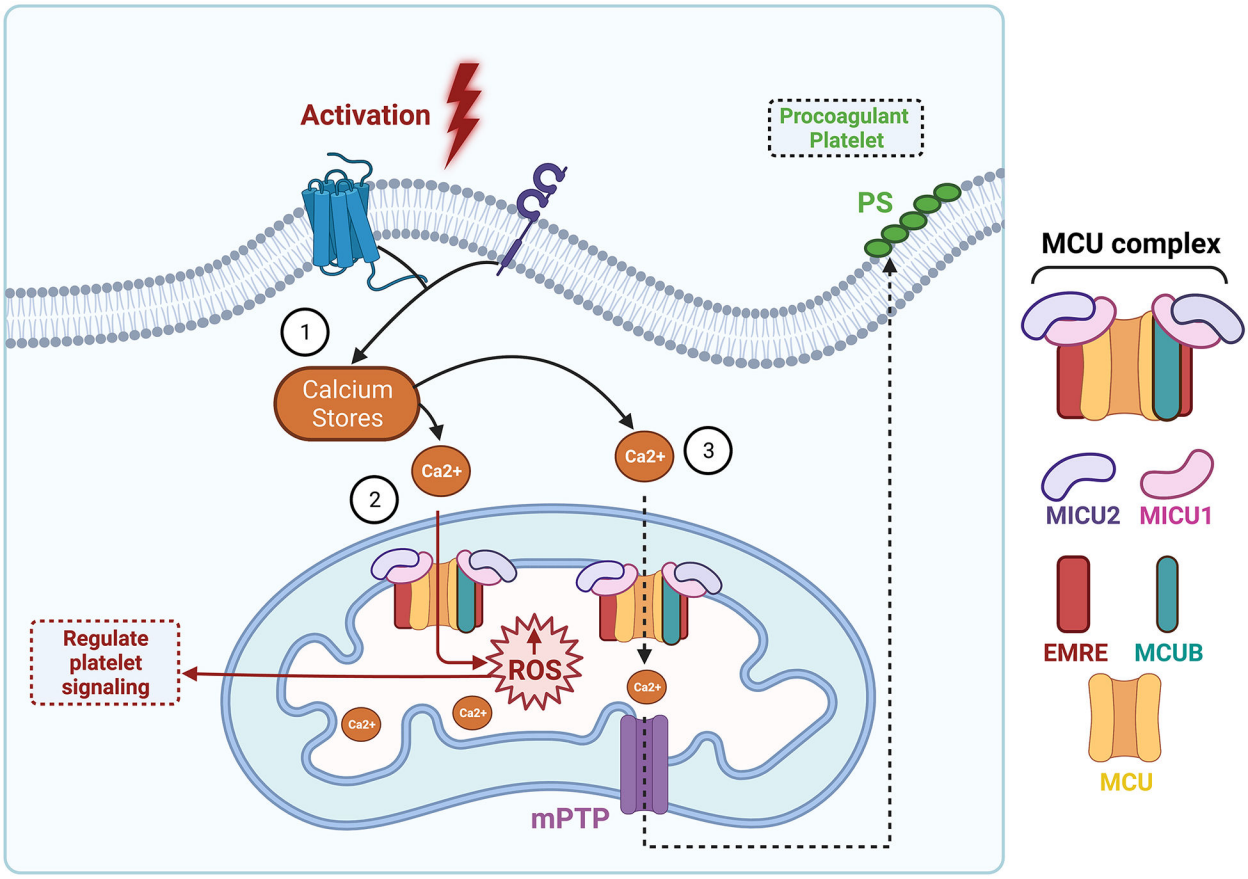

Figure 2. Platelet activation induces the formation of mitochondrial ROS and the formation of procoagulant platelets.

(1) Platelet activation triggers the opening of intracellular Ca^{2+} stores. (2) This results in mitochondrial Ca^{2+} uptake through the MCU (mitochondrial calcium uniporter) complex. Platelet activation requires ATP generation. In the process of producing ATP mitochondrial ROS is generated as a byproduct, which can potentiate platelet activation. (3) When mitochondrial Ca^{2+} levels reach a critical threshold, mitochondria will form the mitochondrial permeability transition pore (mPTP) resulting in procoagulant platelet formation.