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Increased platelet storage time is associated with mitochondrial dysfunction and impaired platelet function

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Abstract

Background—Hemorrhagic shock is a leading cause of death following severe trauma and platelet transfusions are frequently necessary to achieve hemostasis. Platelets, however, require special storage conditions and storage time has been associated with loss of platelet quality. We hypothesized that standard storage conditions have a deleterious effect on platelet mitochondrial function and platelet activation.

Materials and methods—Platelet donations were collected from healthy donors (n=5), and stored in gas permeable collection bags according to American Association of Blood Bank recommendations. Platelet units were sampled from day of collection (day 0) until day 7. High resolution respirometry was used to assess baseline mitochondrial respiration, maximal oxygen utilization, and individual mitochondrial complex-dependent respiration. FACS was performed to analyze mitochondrial content, mitochondrial reactive oxygen species (ROS), the expression of P-selectin (both before and after challenge with thrombin receptor activating peptide (SFLLRN)), and apoptosis. Data was analyzed using ANOVA and Pearson's correlation ($p < 0.05$ significant).

Results—Mitochondrial respiration decreased significantly in platelets stored longer than 2 days ($p < 0.05$). Platelets also demonstrated a persistent decrease in response to stimulation with

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SFLLRN by the 3rd day of storage ($p<0.05$) as well as an increase in mitochondrial ROS and apoptosis ($p<0.05$). Mitochondrial respiration significantly correlated with platelet capacity to activate ($r=0.8$, $p<0.05$).

Conclusion—Platelet mitochondrial respiratory function and activation response decrease significantly in platelets stored for 3 or more days. Because platelet transfusions almost universally occur between the 3rd and 5th day of storage, our findings may have significant clinical importance and warrant further *in vivo* analysis.

Introduction

Following severe traumatic injury, patients are frequently coagulopathic on admission and death from acute blood loss typically occurs within the first 6 hours post-injury.(1–3) In addition, to definitive control of bleeding, treatment of ongoing hemorrhage requires the transfusion of blood products in order to achieve hemostasis and stabilization. Recently, transfusing platelets in a 1:1:1 ratio with packed red blood cells and plasma has been shown to improve mortality.(2) Although trauma patients have benefited enormously from the early use of platelet transfusion (4); the supply does not match a growing demand.(5) Overall, the national blood supply is decreasing as a result of increased medical and surgical demand, decreased blood donation rates, and increasingly stringent FDA blood safety regulations.(6) Currently, platelet storage is limited to only 5 days by FDA regulations, although the normal *in vivo* life span of platelets is 8 to 10 days.(7, 8) Methods to improve and extend platelet shelf life could have an enormous impact on improving platelet availability.

Unlike other blood products, platelets require unique storage conditions in order to maintain function and viability. Given the risk of premature activation, platelets intended for transfusion must be stored at room temperature, in gas permeable bags and with constant agitation.(9) Despite these current practices, over time stored platelets develop a “platelet storage lesion” that is characterized by a gradual decline in activation, aggregation and degranulation – all processes that are highly energetic and necessary for adequate hemostatic function.(10,11) Moreover, given that storage time has been shown to increase reactive oxygen species production as well as enhance apoptosis,(12,13) we hypothesized that increased platelet storage time would directly correlate with mitochondrial dysfunction and the subsequent development of impaired platelet activation. A better understanding of how storage time impacts mitochondrial bioenergetics and platelet function could potentially lead to new strategies to prolong shelf-life; thus, improving the supply of this critical blood component.

Materials and Methods

Platelet collection

Following approval from the Institutional Review Board at the University of Pennsylvania, platelet donations were obtained from healthy volunteers (ages 18–65 years). Volunteers were excluded if they were taking non-steroidal anti-inflammatory agents or steroids, if they had a history of easy bruisability or known platelet disorder including thrombocytopenia, or if they had chronic renal insufficiency. After obtaining informed consent, approximately 150 mL of blood was collected passively by standard venipuncture (18 gauge) and anticoagulated using Acid-Citrate-Dextrose (ACD-A) in a 12:1 ratio. Using centrifugation (250 x rcf for 12 minutes) at least 60 – 80 mL of platelet-rich plasma (PRP) was obtained from each donor with a total platelet count of at least 10^6 – 10^8 cells/mL per donation. PRP units were stored in highly gas permeable storage bags (TrimaAccel, Terumo BCT, Inc., Lakewood, CO) at room temperature without agitation for 1 hour to reduce platelet activation. Units were then stored on a flatbed agitator (Helmer, Noblesville, IN; 65–70

cycles/min) at $22 \pm 2^\circ\text{C}$ according to American Association of Blood Banks (AABB) standards.(14,15) Platelets were sampled on Days 0, 1, 2, 3, 4, 5 and 7 using aseptic technique via a sterile connecting device (Terumo Medical Corp., Somerset, NJ).

Platelet mitochondrial respiration

Platelet mitochondrial respiratory function was assessed using high-resolution respirometry (Oxygraph-2k Oroboros Instruments, Innsbruck, Austria).(16) Platelets were suspended in a 2mL glass chamber at concentration of 2×10^8 cells/ml in respiration medium B (RM-B) containing mannitol (110 mM), EGTA (0.5 mM), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (3 mM), Taurine (20 mM), KH_2PO_4 (10 mM), K-lactobionate (60 mM), dithiothreitol (DTT, 0.3 mM) and 0.1% Fatty acid-free bovine serum albumin (FFA-BSA) at pH of 7.1.(17) Platelets were allowed to stabilize at routine respiration without exogenous substrates in RM-B at 37°C ; platelets were then permeabilized with digitonin ($1 \mu\text{g}/1\text{L} \times 10^6$ platelets). Complex I-dependent respiration (CI State 3 Respiration) was evaluated by adding malate (5 mM), pyruvate (5 mM), ADP (1 mM), and finally glutamate (5 mM). Maximal oxidative-phosphorylation capacity through both Complex I and Complex II (CI+CII State 3) was obtained by subsequently adding succinate (10 mM). State 4 Respiration was then evaluated by adding oligomycin, an ATP-synthase inhibitor ($1 \mu\text{g}/\text{mL}$). Maximal capacity of the electron transport system (CI+II ETS capacity) was obtained by titrating the protonophore carbonyl cyanide p-trifluoromethoxy phenylhydrazone (FCCP) in $2 \mu\text{M}$ increments until respiration reached a plateau. Complex I was then inhibited by rotenone ($1 \mu\text{M}$) in order to assess maximal ETS capacity via Complex II alone (CII ETS). Finally, residual oxygen consumption was determined by adding antimycin-A ($2.5 \mu\text{M}$), a complex III inhibitor. The Respiratory Control Ratio (RCR) was calculated (i.e. Complex I State3/State 4) in order to evaluate the relationship between mitochondrial respiration and ATP synthesis. Oxygen concentration and oxygen flux were acquired and analyzed in real-time (DatLab software 4.3, Oroboros Instruments, Innsbruck, Austria).

Platelet mitochondrial content and reactive oxygen species

Platelet mitochondrial content and reactive oxygen species (ROS) were measured using fluorescence-activated cell sorting (FACS). Briefly, platelet samples were resuspended in Tyrode's buffer consisting of HEPES (10 mmol/L; pH 7.4), NaCl (134 mmol/L), NaHCO_3 (12 mmol/L), KCl (2.9 mmol/L), Na_2HPO_4 (0.34 mmol/L) and MgCl_2 (1 mmol/L) to a concentration of 10^6 cell/mL. To determine the mitochondrial content, platelets were incubated with MitoTracker green (MTG, Life Technologies, New York, NY) in diluted Tyrode's buffer (100 nM) for 30 minutes at 37°C . Production of mitochondrial reactive oxygen species was determined by incubating platelet samples diluted in Tyrode's buffer with 2-hydroxyethidium (MitoSox Red (MSR) $5 \mu\text{M}$, Life Technologies, New York, NY) at 37°C for 10 minutes. Using 515/20 and 660/20 filters for MTG and MSR respectively, data was acquired using a LSR II-A flow cytometer (BD, Bioscience, San Jose, CA) with DiVa 6.1.2 software (BD Bioscience, San Jose, CA). For each sample, only cells that stained positive for MTG were included for analysis and 100,000 events were recorded. Data was analyzed using FlowJo software (Tree Star, Inc, Ashland, Oregon).

Assessment of Platelet Activation

Platelet basal activation was determined using FACS. Platelets were resuspended in Tyrode's buffer and co-incubated with Pacific Blue conjugated anti-CD41 antibodies to identify platelets as well as allophycocyanin (APC) conjugated anti-CD62P (P-selectin) antibodies to detect platelet activation (BioLegend, San Diego, CA). Platelets were incubated with both antibodies for 25 minutes at room temperature in the dark. To assess the ability to activate upon stimulation, platelets were continuously mixed with $10 \mu\text{M}$ of thrombin-receptor-derived hexapeptide (SFLLRN) at room temperature for 5-10 minutes

and then co-incubated with anti-CD41 and anti-CD62P antibodies as previously described. Stained platelets were fixed with 2% paraformaldehyde and analyzed by FACS. A violet laser and 450/50 filter were used to measure Pacific Blue. A red laser and 660/20 filter were used to assess APC.

Assessment of platelet apoptosis

The expression of membrane phosphatidylserine was used as a marker for apoptosis. APC-conjugated annexin V was used to detect the presence of phosphatidylserine (eBioscience, Inc. San Diego, CA). Platelet viability was confirmed using 7-actinomycin C (7-AAD, eBioscience, Inc. San Diego, CA). In brief, platelets ($\sim 10^8$) were incubated in the dark with APC-Annexin V ($\sim 5 \mu\text{g/mL}$) at room temperature for 15 minutes followed by 7-AAD ($2.5 \mu\text{g/mL}$). Using flow cytometry, APC was detected using a red laser and a 660/20 filter and 7-AAD was assessed using blue laser with a 695/40 filter. Again, 100,000 events were recorded and analyzed as previously described.

Statistical analysis

Data analyses were performed using SPSS 19.0 software, (Armonk, New York). One-way analysis of variance (ANOVA) was used to compare data across multiple time points. After assessing for homogeneity of variance, Bonferroni and Tamhane corrections were applied post-hoc as appropriate. Pearson's correlations were performed to assess the relationship between changes in mitochondrial respiration and differences in platelet activation over time. A p value of less than 0.05 was considered statistically significant.

Results

Platelet mitochondrial respiration

Stored platelets experienced a significant and persistent decrease in all measured respiratory capacities within the first two days of storage. Basal respiration fell from 45.32 ± 3.82 to 10.66 ± 2.09 ($\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets) by the 2nd day of storage and remained significantly depressed over the next 5 days ($p < 0.05$, Fig. 2). Complex I state 3 respiration also experienced a significant decline of $\sim 50\%$ by the 1st day falling from a basal value of 99.87 ± 18.65 to 48.53 ± 9.34 ($\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets). Complex I state 3 continued to decline, falling to 17.9 ± 5.02 ($\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets) by the 2nd day ($p < 0.05$) and remaining significantly depressed through day 7. Similarly, Complex I+II state 3 respiration declined significantly within the first two days of storage (153.45 ± 22.83 vs. 35.75 ± 7.34 $\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets; $p < 0.05$) with lowest levels observed on the 7th day. Oligomycin induced state 4 respiration experienced a significant and persistent decline by the 1st day of storage (24.68 ± 5.29 vs. 14.54 ± 0.67 $\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets; $p < 0.05$) and remained suppressed until day 7. FCCP-induced maximal respiration also fell 50% by the first day of storage (171.26 ± 25.69 vs. 68.32 ± 11.8 $\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets, $p < 0.05$), continued to decline by the 2nd day (26.79 ± 7.22 $\text{pmol O}_2/\text{s} \times 10^{-8}$ platelets, $p < 0.05$) and remained persistently depressed until day 7. Finally, the respiratory control ratio (RCR) decreased roughly 75% by the 2nd day of storage (4.56 ± 0.89 vs. 1.23 ± 0.26 , $p < 0.05$) and remained suppressed for the remainder of the experiment. (Fig. 2)

Production of ROS and Mitochondrial content

After controlling for mitochondrial content, the production of mitochondrial based ROS was noted to increase significantly over time ($p < 0.05$). By the 3rd day of storage, ROS production was 18 times higher than that at baseline. ROS production peaked on the 5th day of storage reaching $38.86 \pm 3.93 \%$ ($p < 0.05$). Interestingly, the levels of ROS decreased on the 7th day and were similar to those measured on the 1st day. (Fig. 3)

Platelet response to activation

The response of platelets to a strong activator (SFLLRN) was significantly higher in fresh platelets (Day 0) when compared to all other time points ($p<0.05$). By day 3, platelets demonstrated a profound decrease in their capacity to activate appropriately ($39.87 \pm 11.47\%$ vs. $2.43 \pm 0.79\%$, $p<0.05$) and remained dysfunctional through day 7 (Fig. 4)

Apoptosis and Platelet Storage Time

We found a significant increase in the expression of phosphatidylserine within the first few days of storage (Fig. 5). By the 3rd day, this marker of apoptosis was 6x higher than that observed on the 1st day (1.36 vs. 6.15% , $p<0.05$). Apoptosis peaked by the 7th day storage ($9.83 \pm 1.27\%$). Importantly, we did not observe any significant change in platelet viability as measured by 7-AAD (data not shown).

Mitochondrial respiration correlates with the response to activation

There was a strong and positive association between mitochondrial respiration and the platelet response to activation. In particular, platelet activation significantly correlated with basal respiration ($r=0.86$, $p<0.05$), complex I state 3 respiration ($r=0.93$, $p<0.05$), complex I +II state 3 respiration ($r=0.94$, $p<0.05$), state 4 respiration ($r=0.7$, $p<0.05$), maximal respiration of complex I+II ($r=0.94$, $p<0.05$) and maximal respiration of complex II ($r=0.81$, $p<0.05$). (Fig. 6) There was no correlation between mitochondrial respiration and the production of ROS or expression of annexin V. Additionally; there was no correlation between the ROS production and the response to activation (Data not shown).

Discussion

Mitochondrial bioenergetics plays a critical role in maintaining platelet function. Indeed, mitochondrial generated ATP provides the major source of energy used for maintaining platelet cellular integrity and function both *in vivo* and during storage.(9, 15, 18, 19) In this study, we investigated the relationship between mitochondrial function and the capacity for platelet activation as a function of storage time. Our research demonstrates that stored platelets experience a dramatic decrease in mitochondrial respiration in conjunction with increased ROS production, enhanced apoptosis, and decreased functional capacity. Importantly, the observed decline in platelet respiratory and cellular function began within the first 2 days of storage. Because platelets require a detailed screening and preparation period, platelets intended for transfusion are not currently released by the blood bank before the 3rd day of storage. As such, our data suggests that platelet units routinely used clinically may potentially be dysfunctional at the time of transfusion.

The bioenergetic state of stored platelets is vitally dependent on competent mitochondrial respiration. Because ATP is synthesized more efficiently by oxidative phosphorylation than by anaerobic glycolysis, platelet function is contingent on maintaining mitochondrial performance. Storage time, however, significantly reduces mitochondrial function. In a study investigating platelet storage and mitochondrial capacity, Diab et al. (7) found that both platelet cytochrome C oxidase activity and ATP content decreased progressively during storage. Our research also reveals a negative association between platelet storage time and mitochondrial bioenergetics. In our study, all parameters of mitochondrial oxygen consumption fell significantly within the 1st day of storage and nadired at 75% of baseline within 48 hours of storage. Importantly, the respiratory control ratio – a measure of oxidative phosphorylation efficiency - also dramatically decreased within the first day of storage. The respiratory control ratio further declined to values approaching ~1 by the 2nd day of storage. This suggests that aerobic mitochondrial ATP synthesis was totally

suppressed within 48 hours of storage and may account for the depleted ATP levels observed in previous reports.(7, 17, 20)

Platelet storage may independently increase the production of ROS.(12) Periods without agitation are associated with hypoxia in banked platelets and decreased oxygen tension has been shown to stimulate the production of mitochondrial-based ROS.(13,21) Moreover, the increased generation of free radical oxygen species during hypoxia can further stimulate the production of ROS by cytosolic NADPH oxidase. This increase in ROS may trigger the opening of mitochondrial ATP-sensitive potassium channels causing more ROS production and thereby perpetuating a vicious circle.(22–24) Increased ROS production can also lead to the formation of the mitochondrial permeability transition pore, loss of cytochrome C, and enhanced apoptosis.(13) On the other hand, ROS production may be a key intracellular regulator in platelet activation with NADPH oxidase playing a prominent role.(22, 23) In our study, mitochondrial based ROS production progressively increased during storage and was significantly higher than baseline values on the 3rd and 5th day of storage. Interestingly, there was a dramatic decrease in the mitochondrial production of ROS on the 7th day- a phenomenon that may be related to almost total inhibition of the mitochondrial activity observed on day 7.(25) Although we did not measure NADPH oxidase activity in our study, the regulatory role and interactions between the mitochondrial production of ROS and the NADPH oxidase system clearly warrant further attention.

Current storage conditions may also promote platelet apoptosis. Although apoptosis is a mechanism of programmed cell death in nucleated cells, several studies have indicated that platelets may also undergo this process despite the fact that they are anucleate cellular fragments.(8, 11, 26) In this study, we measured phosphatidylserine expression as a proxy for apoptosis. While phosphatidylserine expression has been used as a marker for platelet activation in stimulation studies, its expression in stored platelets is closely associated with the development of apoptosis and platelet dysfunction.(11, 15) With storage, phosphatidylserine expression correlates with several key apoptotic events including the release of cytochrome C, activation of caspase-3 and stimulation of Rho associated kinase-1.(27) In our study, we noted a significant increase in phosphatidylserine expression within three days of storage. Because platelets expressing phosphatidylserine are rapidly removed from the circulation, platelets stored under current conditions may also be at risk for prompt clearance *in vivo*, thus, decreasing the overall efficacy of the transfused platelet unit.(28) Decreasing the expression of phosphatidylserine by improving the current storage conditions may lead to increase the platelet survival *in vivo* and therefore improved overall function.

Storage time not only increases the risk of premature platelet activation, it may negatively impact platelet functional activity overall.(5, 7, 9,19) In our study, we tested platelet function in stored units by using a strong thrombin receptor stimulator (SFLLRN) to induce platelet activation. By the 3rd day of storage, only ~2 % of platelets were able to respond appropriately. Given that storage time also negatively impacted mitochondrial respiration, we hypothesized that the observed decrease in activation response may be secondary to impaired mitochondrial function. In fact, we found a significant and strong correlation between all measurements of mitochondrial respiration and platelet response to activation; suggesting that mitochondrial well-being may be essential in preserving platelet quality during storage.(8)

While our study suggests that stored platelets experience significant mitochondrial and functional abnormalities, the clinical impact of platelet storage time needs to be clarified. In a number of clinical studies, stored platelets appear viable and regain function after transfusion when measured by radioisotope labeling and flow cytometry.(29, 30) Moreover, there does not appear to be an association between the age of banked platelets and outcomes

in adult patients following elective cardiac surgery.(31) That being said, many severely injured trauma patients present with altered platelet function and a consumptive coagulopathy.(32–34) It is unclear if stored platelets are less functional or are at increased risk for clearance in this critically ill population. Although the use of “fresh” platelets has not been directly investigated, it is interesting to note that the use of fresh whole blood, which includes fresh platelets, has been associated with better hemostasis and improved platelet function. In a study of pediatric patients undergoing open heart surgery, Manno et al demonstrated that patients receiving fresh whole blood (24–48 hours old) had significantly less intra and post-op blood loss as well as better platelet function than did patients who received reconstituted old blood products (3–5 days of storage).(35) Similarly, Gruenwald et al found that fresh reconstituted whole blood (2 days of storage) was associated with better clinical outcomes and significantly less bleeding during cardiopulmonary bypass when compared to standard blood component therapy (platelets 3–5 days old).(36) Finally, Inaba et al demonstrated that severely injured patients treated with platelets near expiration had a significant increase in septic complications.(37) Although intriguing, clearly more prospective studies are needed to clarify whether platelet storage time influences the clinical outcome of critically ill trauma patients.

Our studies, however, have several limitations. First, we used centrifugation to isolate platelets rather than apheresis. In the United States, platelets cannot be purchased or used for research once donated for patient use at a blood bank center. As such, in order to obtain platelet units for research only, apheresis requires special administrative permission and is excessively costly (~\$1,000 per unit). Nonetheless, it has been demonstrated that centrifugation has a similar effect in terms of platelet activation and aggregation when compared to apheresis suggesting these two methods of collection are comparable.(38,39) Moreover, in terms of mitochondrial oxygen consumption, our preliminary results showed that by day 5 of storage, platelets donated by apheresis demonstrated were similar to the units processed by centrifugation. Secondly, the volume of the platelet concentrates were approximately 80 mL per unit, which is greatly different from the standard platelet transfusion unit. Although our volumes never fell below 40 mL, platelets stored at lower volumes may have impaired aggregation *in vitro*.(40) Finally, although measuring activation *ex vivo* may be a good parameter of platelet function, further experiments are necessary to confirm that these findings are clinically important *in vivo*. Microfluidics assay may provide a reliable method of testing platelet behavior in a physiological environment and may be helpful in corroborating our findings.(41)

In conclusion, this study demonstrates that both platelet mitochondrial respiration and function decline as a function of storage time. Furthermore, platelet storage time was associated with increased production of mitochondrial based ROS as well as a progressive increase in apoptosis. In order to improve platelet quality during storage, further research investigating the relationship between mitochondrial function, platelet activation, and clinical outcomes is warranted.

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Figure 1. Mitochondrial respiratory function in permeabilized platelets

Summary of the substrate, uncoupler, inhibitor titration protocol in used to assess mitochondrial respiratory function in permeabilized platelets. Platelets (10^8 cells/ml) were permeabilized with digitonin with subsequent addition of malate and pyruvate. Oxidative phosphorylation (State 3 respiration) was induced by addition of ADP and glutamate as a complex I substrate (CI). The Complex II substrate, succinate, was then added to measure the activity of both complexes. Then, oligomycin, an ATP synthase inhibitor, was added to reveal state 4 respiration (State 4). Maximal respiration was induced by titration of the protonophore carbonyl cyanide p-trifluoromethoxy phenylhydrazone (FCCP). To assess only complex II dependent respiration, rotenone, a complex I inhibitor, was added. Finally, the residual or non-mitochondrial respiration was revealed by addition of a Complex III inhibitor antimycin-A (AA).

Figure 2. Mitochondrial respiratory capacities and Respiratory Control Ratio (RCR)
 A. Mitochondrial respiratory capacities assessed during storage. During storage, platelet experience a significant decrease in all respiratory function including basal respiration, Complex I state 3 respiration, Complex I and II state 3 respiration, oligomycin induced State 4 respiration, and maximal respiration. B. Respiratory Control Ratio (RCR) defined as the ratio of State 3 complex I/State 4 also decreased with storage time, becoming statistically significant on the 2nd day. Values are Mean $\pm$ SEM. $n = 5$, $*p < 0.05$ compared to Day 0.

Figure 3. Platelet mitochondrial production of ROS

After controlling for mitochondrial content using Mito Tracker Green (MTG), platelets incubated in the presence of Mitosox Red (MSR) were analyzed using flow cytometry. A significant increase in the production of mitochondrial based reactive oxygen species (ROS) was observed with increasing storage time. Values are Mean $\pm$ SEM. $n = 5$, $*p < 0.05$ compared to Day 0.

Figure 4. Platelet response to activation and expression of CD62P

Platelet capacity to respond to activation was analyzed using flow cytometry. Platelets were incubated with a thrombin-receptor-derived hexapeptide (SFLLRN) and subsequent activation was assessed by measuring the expression of CD62P. We found a dramatic decrease in the response to activation by the 3rd day of storage that persisted over the next 3 days. Values are Mean $\pm$ SEM. $n = 5$ groups per time point, $*p < 0.05$ compared to Day 0 in CD41 positive cells.

Figure 5. Platelet expression of Phosphatidylserine (PS)

We measured the surface expression of phosphatidylserine during storage by flow cytometry using APC labeled Annexin V. Platelet phosphatidylserine expression significantly increased by the 3rd day of storage and peaked by the 7th day. Values are Mean $\pm$ SEM. $n = 5$ at each time point, * $p < 0.05$ compared to Day 1; § $p < 0.05$ compared to Day 3.

Figure 6. Correlations between mitochondrial respiration and platelet response to activation

Using Pearson's correlations, we analyzed the association between the mitochondrial oxygen consumption and platelet response to activation. A positive and significant correlation was noted between all the respirometry parameters and platelet reactivity ($p<0.05$).