Differential Calcium Signaling and Metabolism in Cold- versus Room Temperature-Stored Platelets

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What do we know about cold temperature (CT) storage of platelets

Advantages of CT storage:

- Shelf-Life Extension: Increased from 5 to 14 days
- Pathogen Reduction: Reduced pathogen load / Decreased pathogen presence

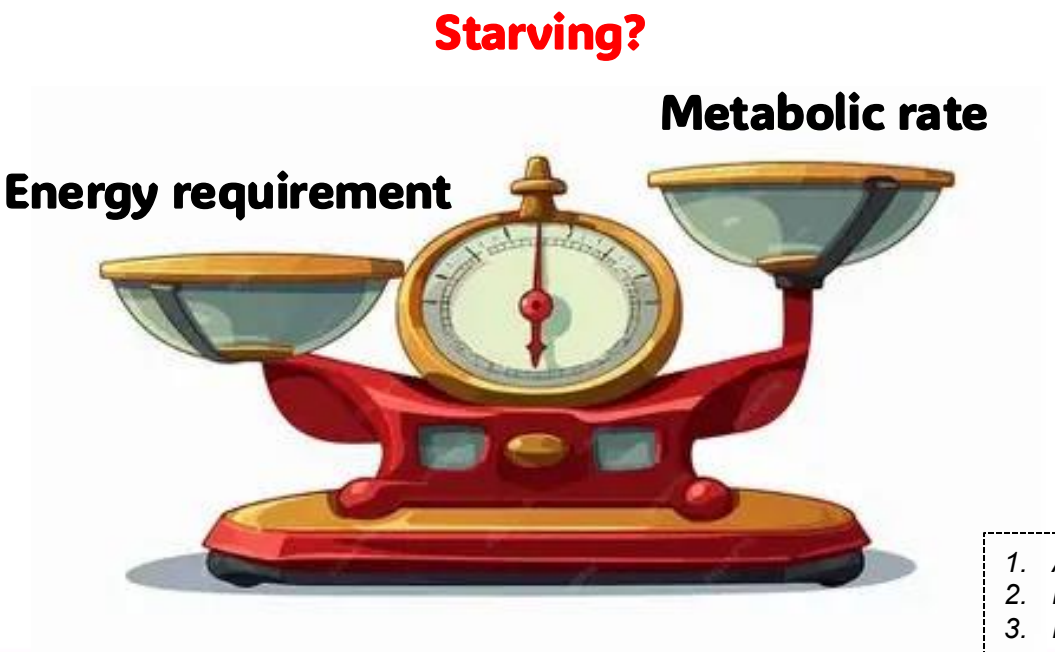
Ongoing clinical trials:

- CHIPS (Chilled Platelet Study): early CSP transfusion is feasible and safe, showing no significant difference in adverse event profiles compared to standard care, also testing efficacy following extended storage (up to 21 days).
- PLTS-1 (Delayed CT-Stored Platelets): Compares delayed cold-stored buffy coat platelets (pathogen-reduced, kept at room temp (RT) for 4 days then transitioned to CT storage for up to 14 days total).

Suppressed metabolism during CT storage: hibernation or starvation?

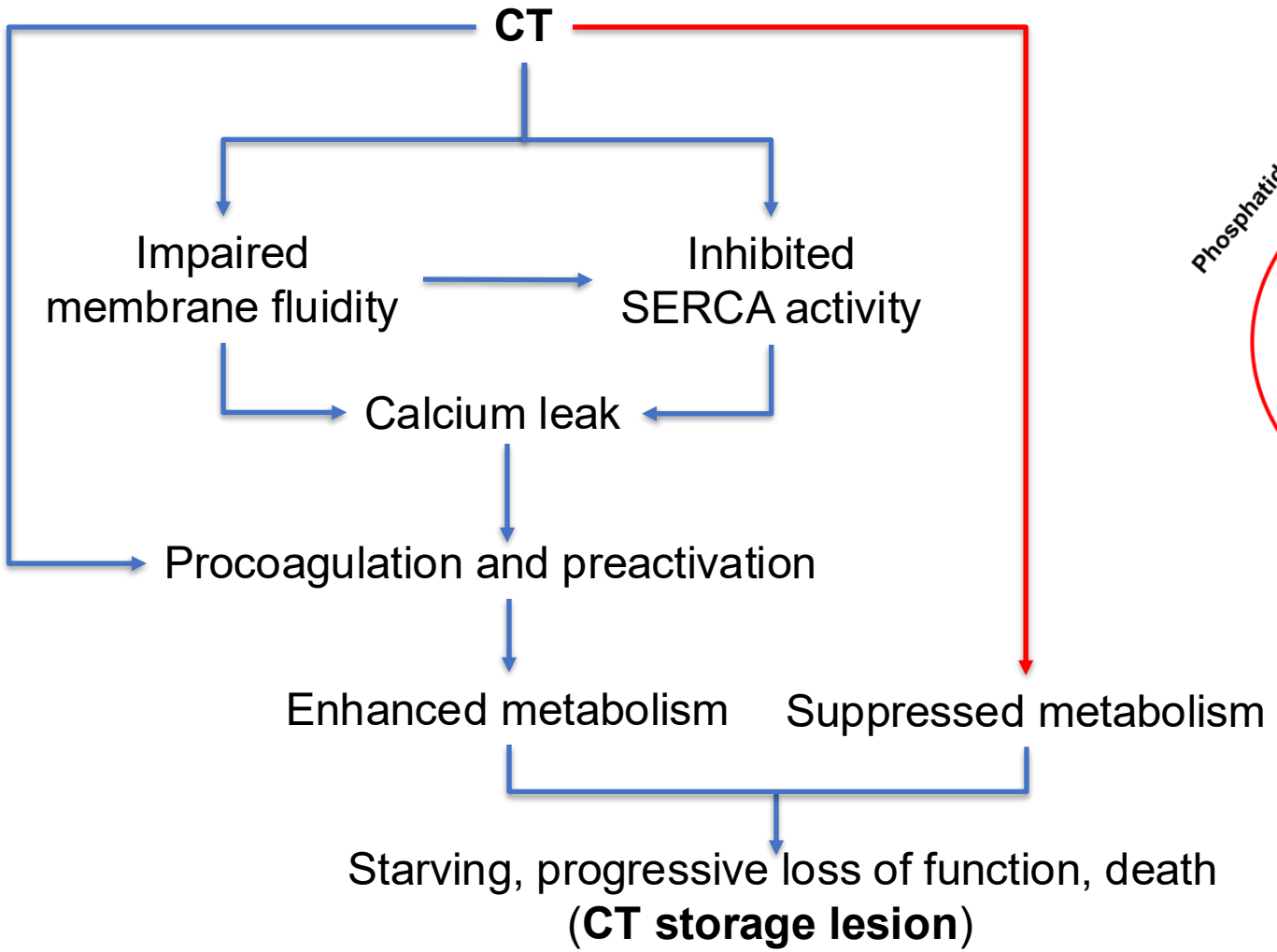
- CT decreases metabolism rate (Q10 TEM coefficient).

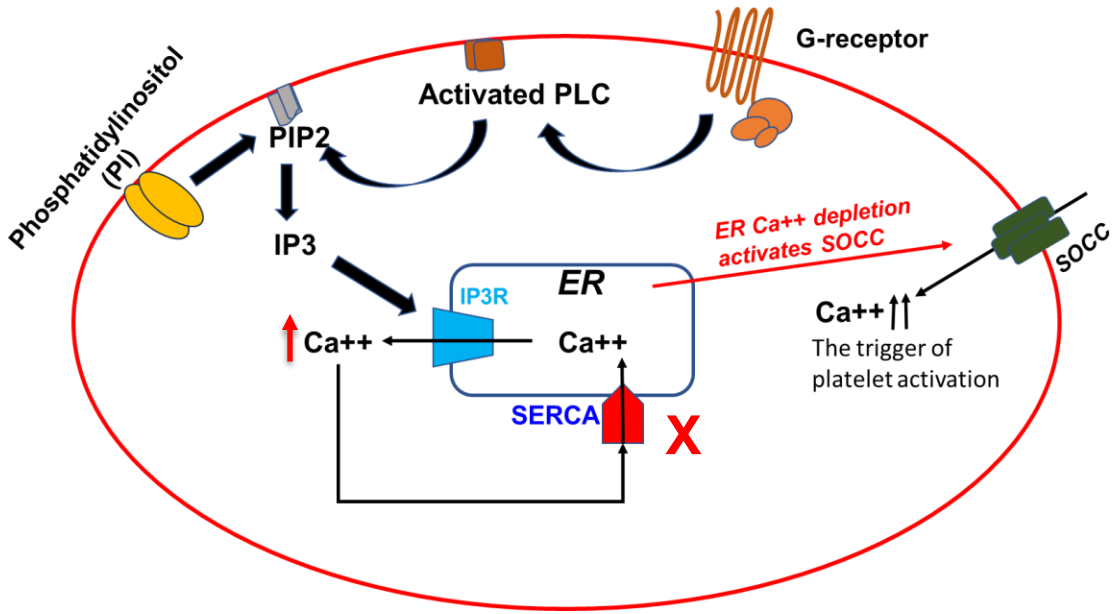
- CT induces early platelet activation: Procoagulation and pre-activation needs > 2-fold ATP, or 3–10-fold increase in glucose uptake. (references 1, 2, 3)



1. *Ajanel., et al. International Journal of Molecular Sciences. 2023.*
2. *Denorme F et al. Am J Physiol Cell Physiol. 2022.*
3. *Kulkarni et al. Haematologia. 2022.*

Hypothesis


CT
Impaired membrane fluidity
Inhibited SERCA activity
Calcium leak
Procoagulation and preactivation
Enhanced metabolism
Suppressed metabolism
Starving, progressive loss of function, death
(CT storage lesion)

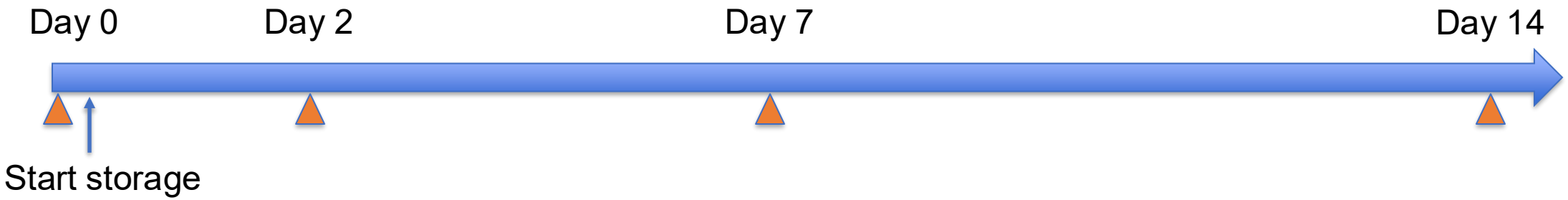

G-receptor
Activated PLC
Phosphatidylinositol (PI)
PIP2
IP3
ER Ca++ depletion activates SOCC
SOCC
IP3R
ER
Ca++
Ca++
Ca++
The trigger of platelet activation
SERCA
X

Methods

Groups:

1. ***PRP-RT:*** Platelet rich plasma (PRP) stored at RT
2. ***PRP-CT:*** PRP stored at CT (4°C)
3. ***PAS:*** PRP diluted platelet additive solution (plasma to PAS: 35 to 65) stored at CT
4. ***DMSO:*** PAS treated with DMSO (1%) stored at CT

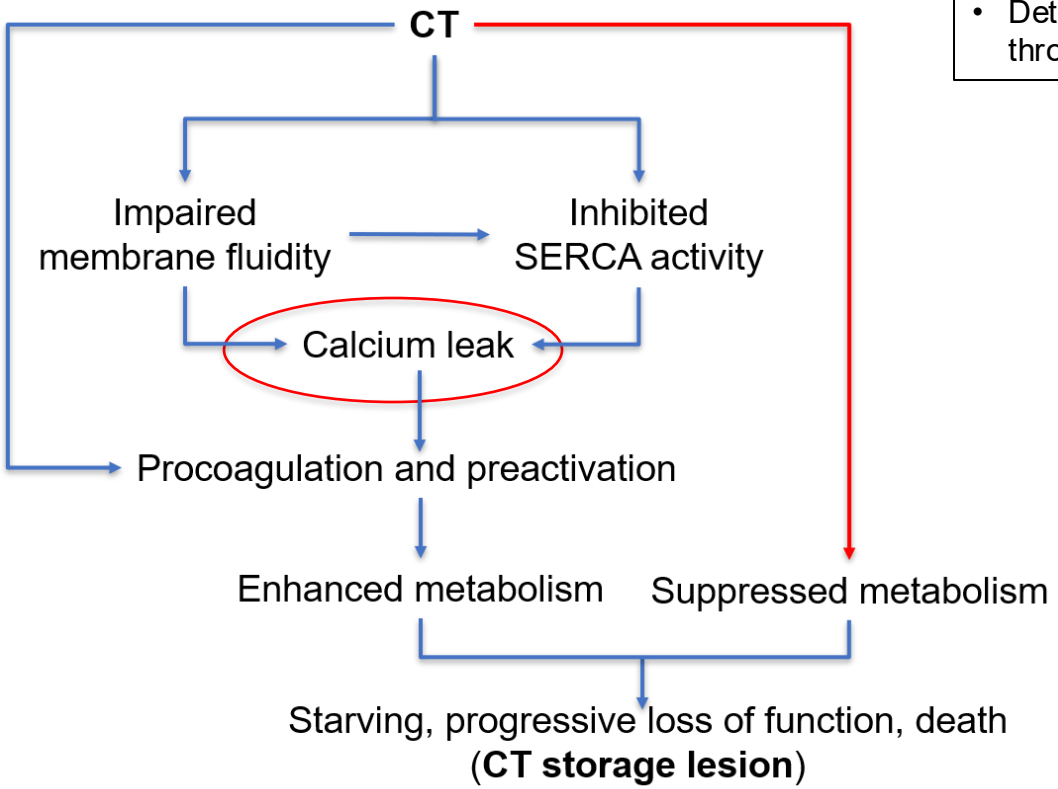
RT	**PRP**
CT	**PRP**
	PAS (plasma dilution)
	PAS+1%DMSO (membrane protection)



Measurements:

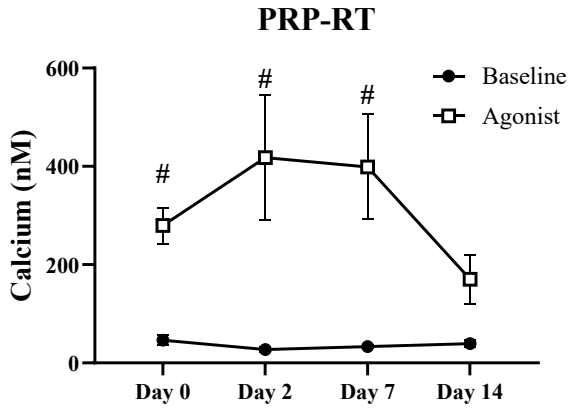
- Cytosolic calcium levels before and after treatment with human thrombin (0.05 U/μL) and convulxin (0.5 μg/μL): flow cytometry (Fluo-3-AM)
- Metabolism (glucose, lactate, electrocytes): iSTAT
- Platelet counts and volume: ADVIA 2000 Analyzer
- Coagulation: thromboelastography (TEG)

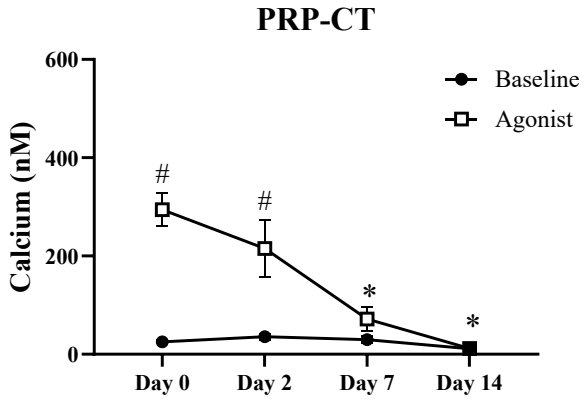
Stored calcium leak during CT storage



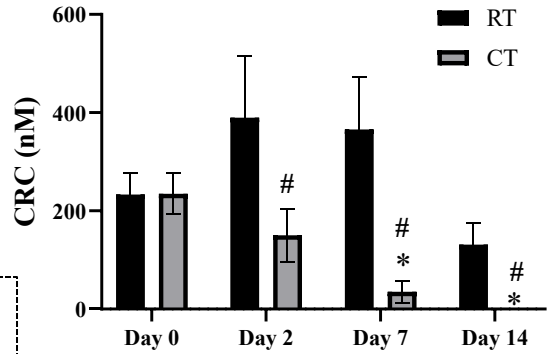
Calcium leak → Impaired CRC

- CRC is the maximal stimulated calcium release capacity.
- Determined by the difference between baseline calcium and the calcium stimulated by agonists thrombin (0.05 U/ml) and convulxin (50 ng/ml).



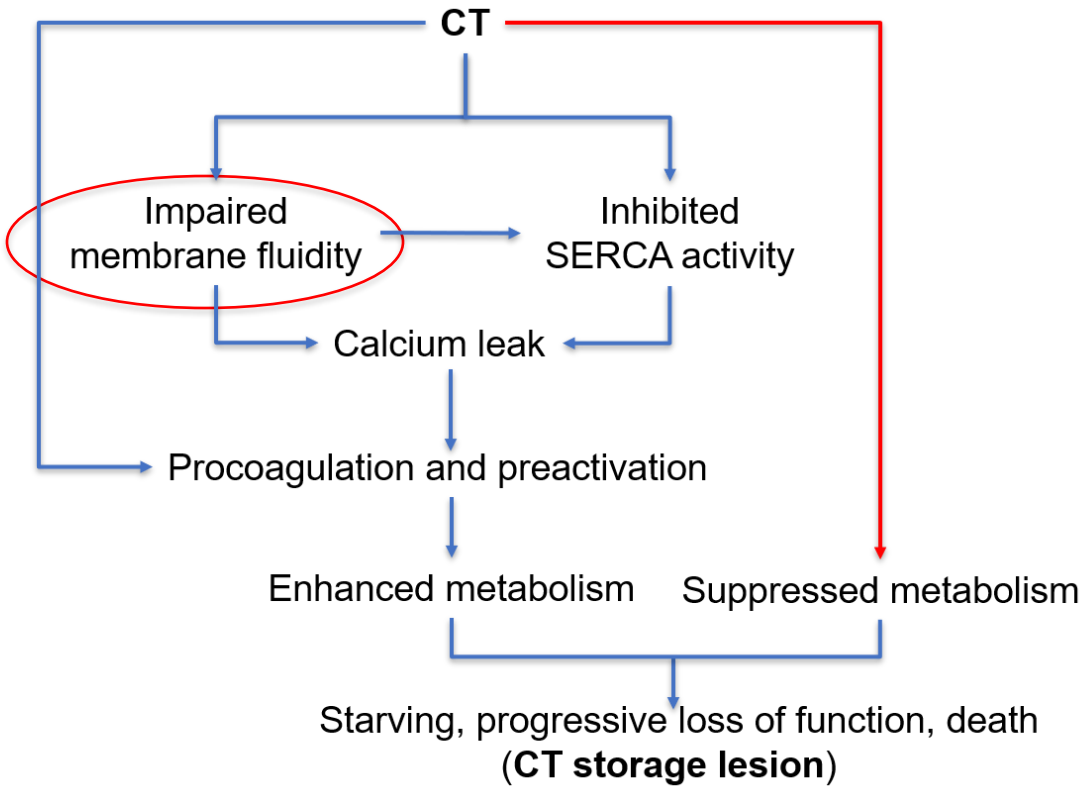


Platelet fatigue **during cold storage**

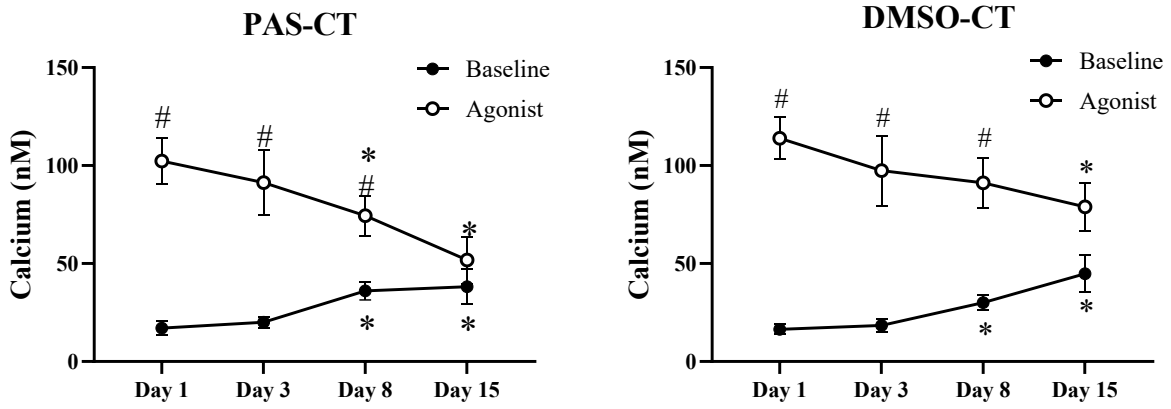


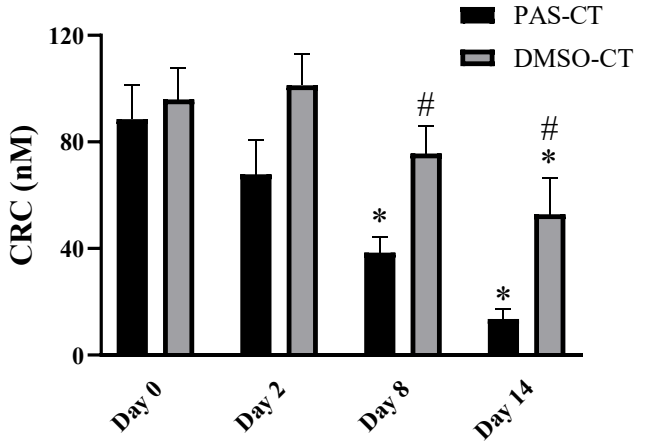
** P < 0.01 vs. Day 0*
 # P < 0.05 between groups

1% DMSO preserved CRC

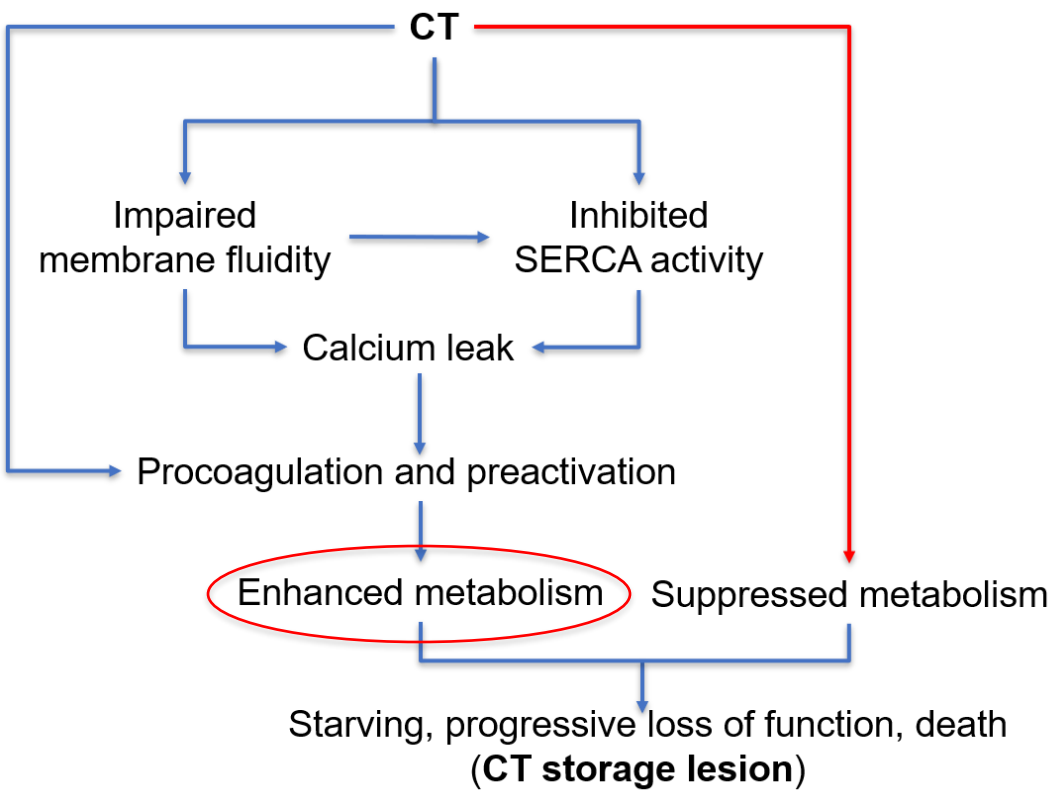


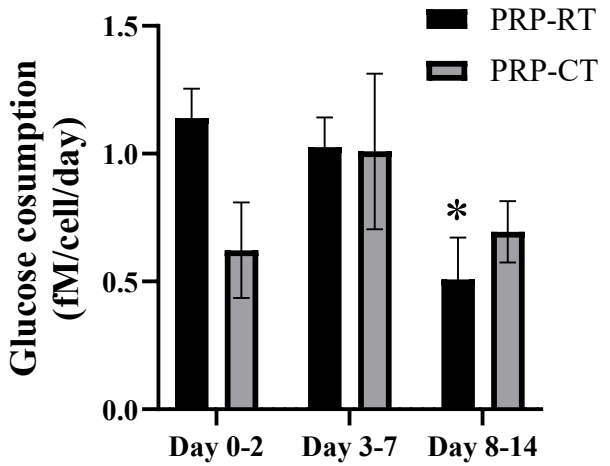
1% DMSO slows down platelet fatigue during CT storage



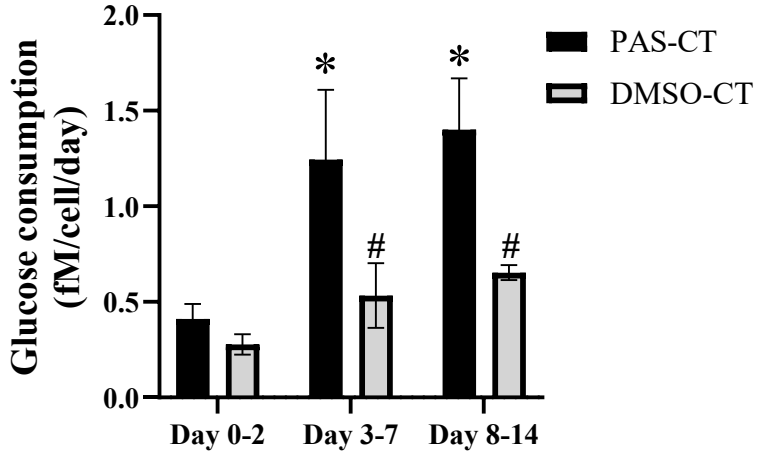


Glucose consumption RT vs. CT

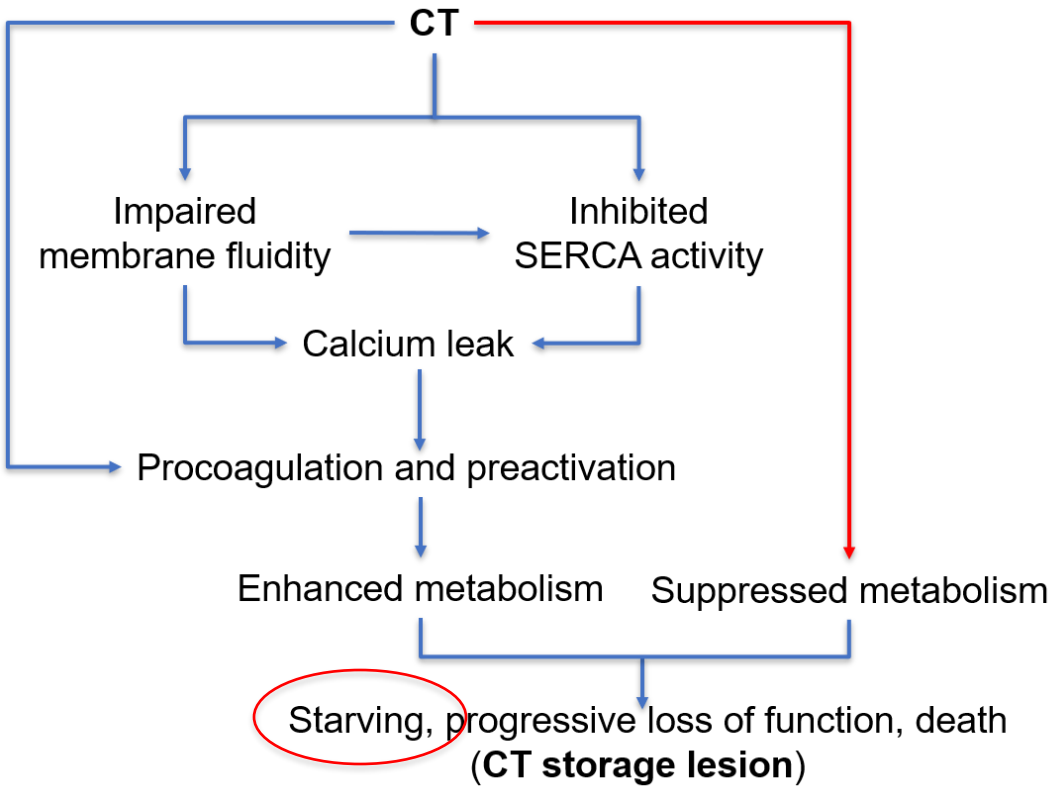




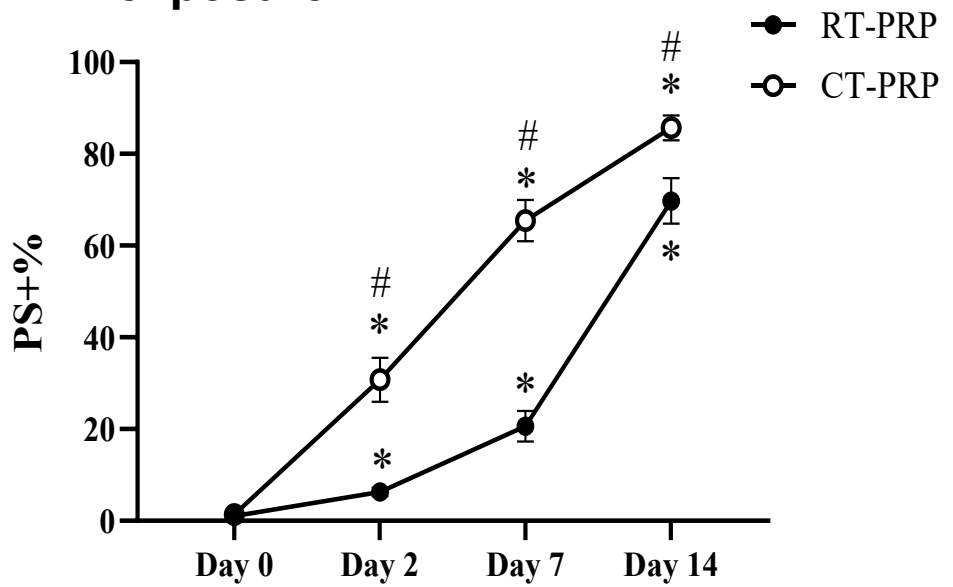
Inhibited glucose consumption in DMSO-CT



PS+ exposure during storage: the marker of "hungry"

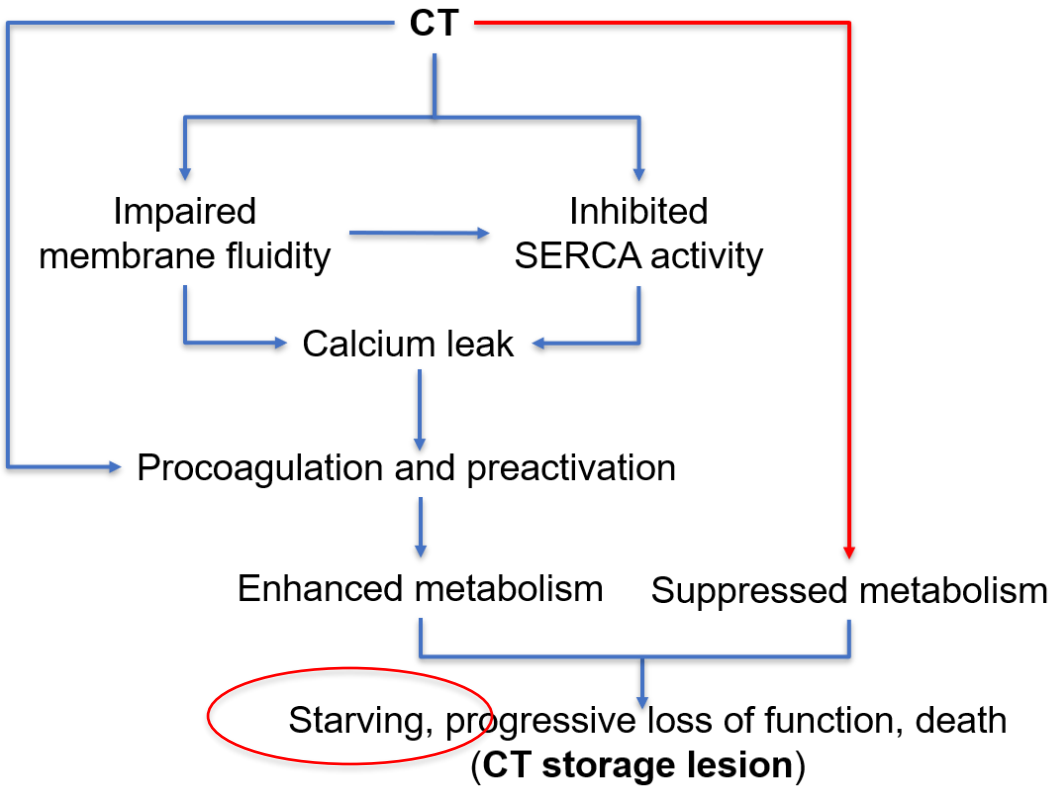


Enhanced phosphatidylserine (PS) exposure in PRP-CT

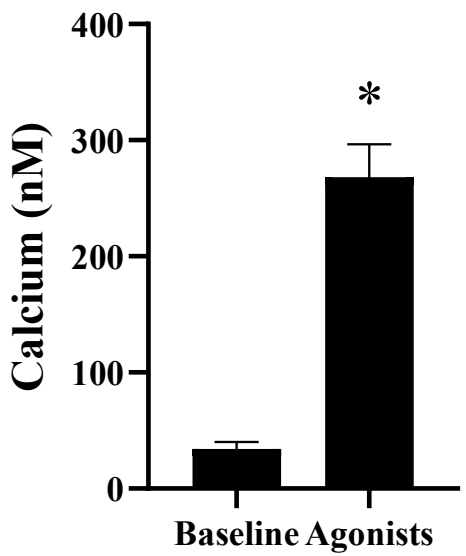


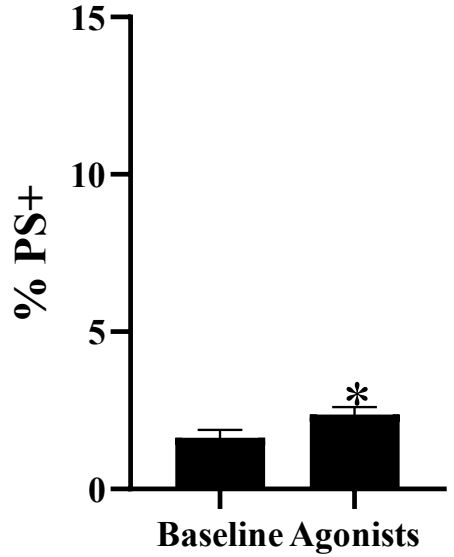
- PS exposure of stored platelets is tightly correlated with availability of ATP
- The cytosol calcium concentration in stored platelets is not high enough to cause PS exposure during storage.

PS+ exposure during storage: the marker of "hungry"



Calcium response and PS+ exposure in **fresh platelets**
(5 min post-agonists)





Key findings and conclusion

- **CT storage is better than RT storage but not perfect yet.**
- **CT-induced Calcium Fatigue:** CT-induced rapid calcium leak and impaired CRC, which is not observed in RT stored platelets.
- **Starvation Over Hibernation:** Cold storage does not purely put platelets into metabolic dormancy; rather, platelets may face starvation due to the sustained energy expenditure to handle the consequences of cold shock and the chronic low-level preactivation.
- This **Metabolic Paradox** may eventually lead to storage lesion (a novel therapeutic direction?).

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Questions

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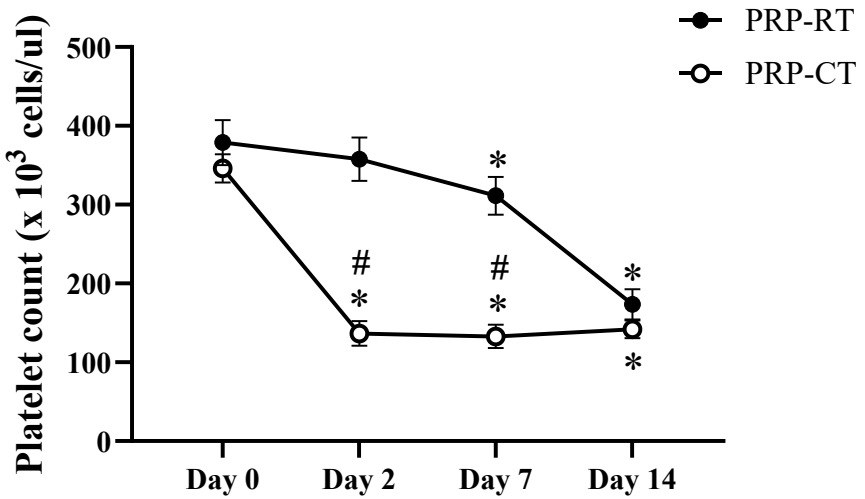
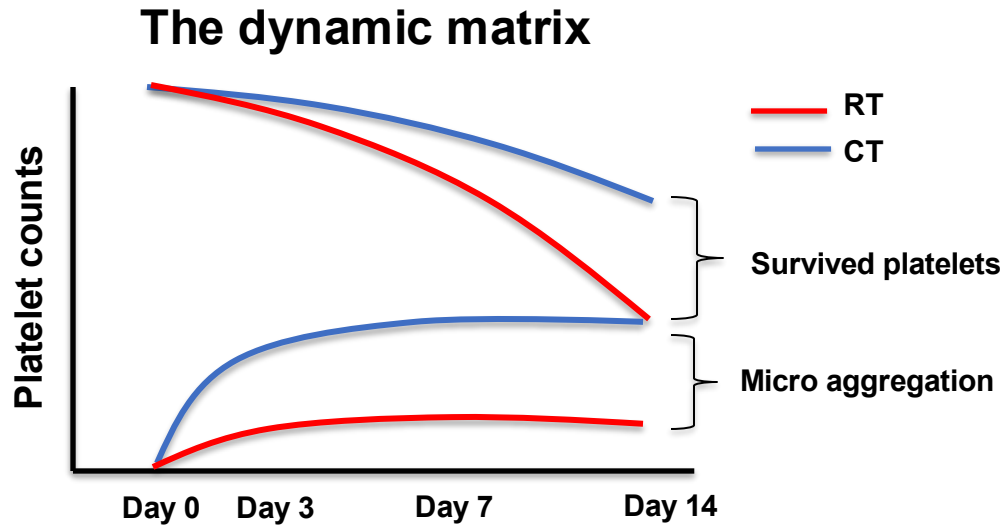
"Military medicine is not just about treating injuries; it's about ensuring the health and readiness of those who serve, so they can accomplish their mission and return home safely."

- MG Paula C. Lodi, Commanding General, USAMRDC

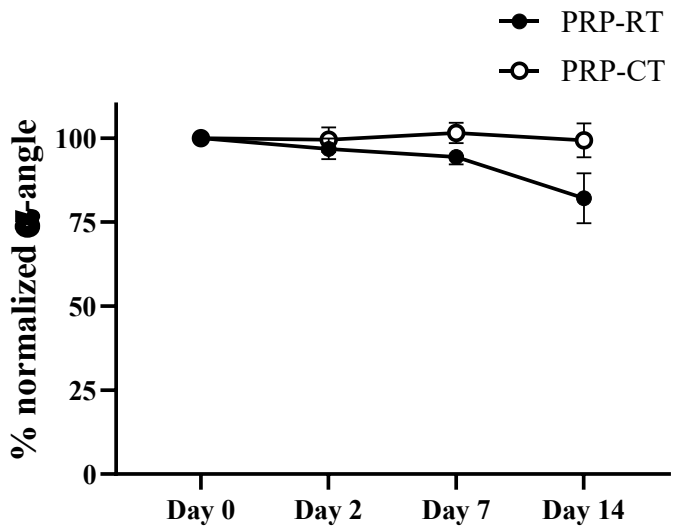
Glucose consumption RT vs. CT

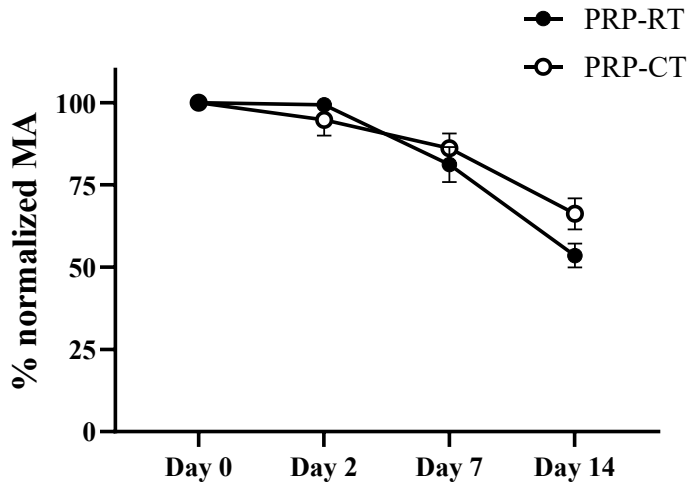




Coagulation RT vs. CT

N =12 for each group





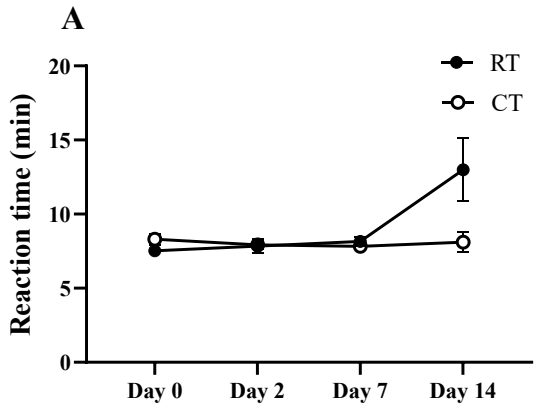
Minimizing the impact from platelet counts using a log-transformation model (ref 1)

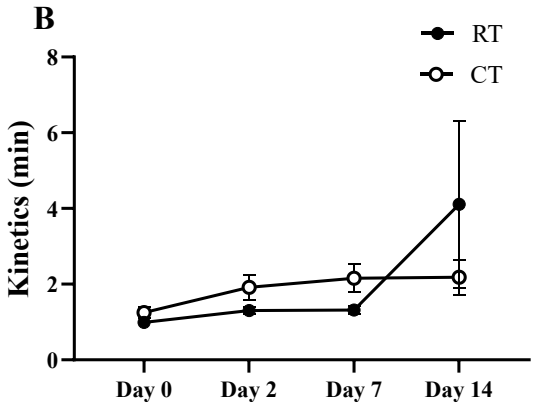
- Normalized a-angle = $MA_0 + 21 \times lg(count_1/count_0)$
- Normalized MA = $MA_0 + 23 \times lg(count_1/count_0)$

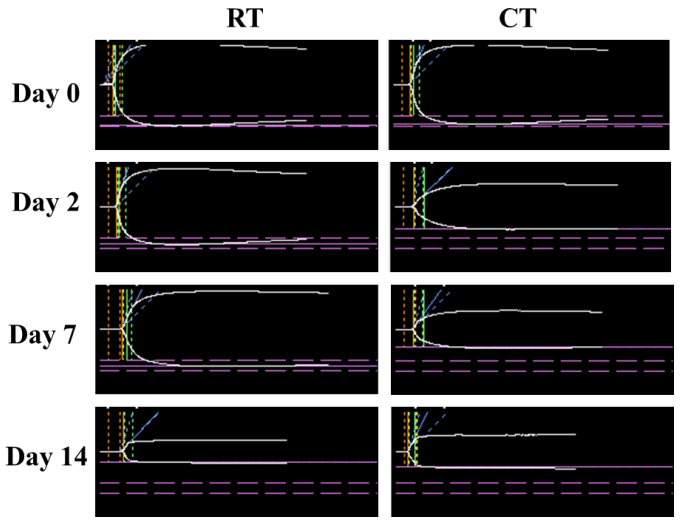
1. Chino K et al. Res Pract Thromb Haemost. 2026 Feb 2;10(2):103378.

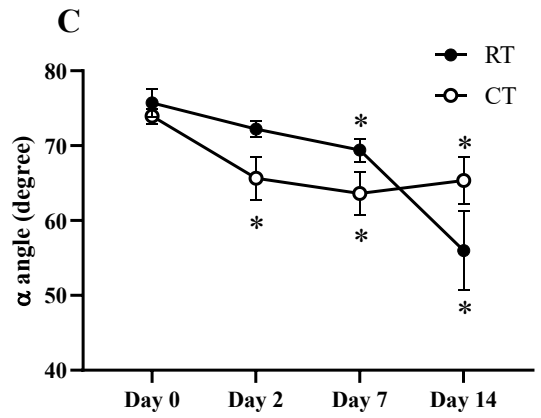
Coagulation RT vs. CT

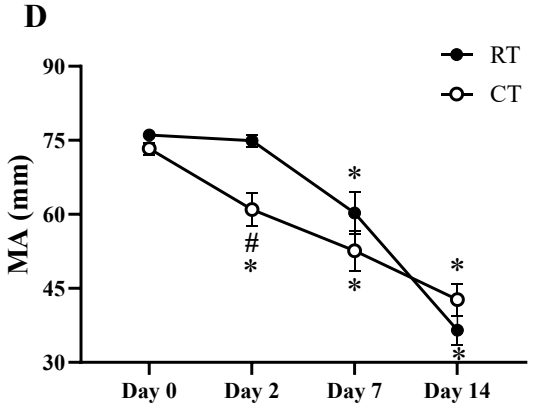
N =12 for each group

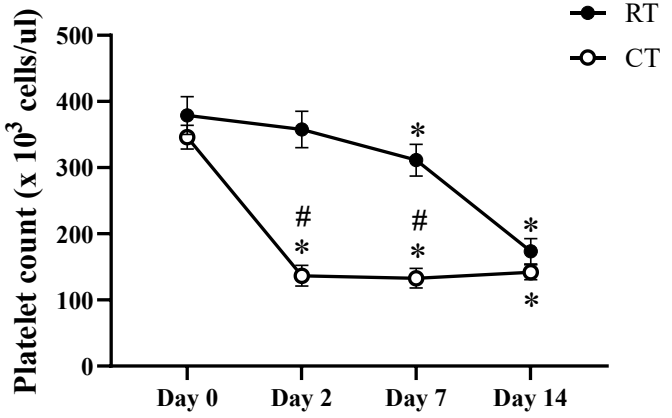




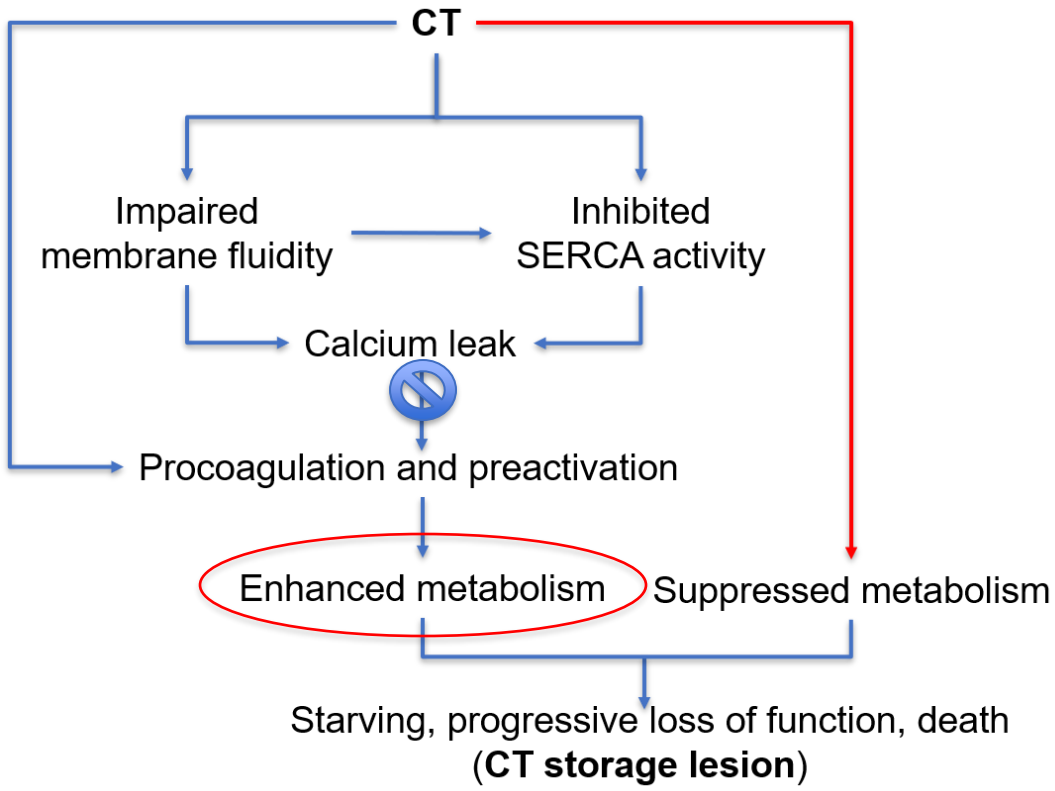








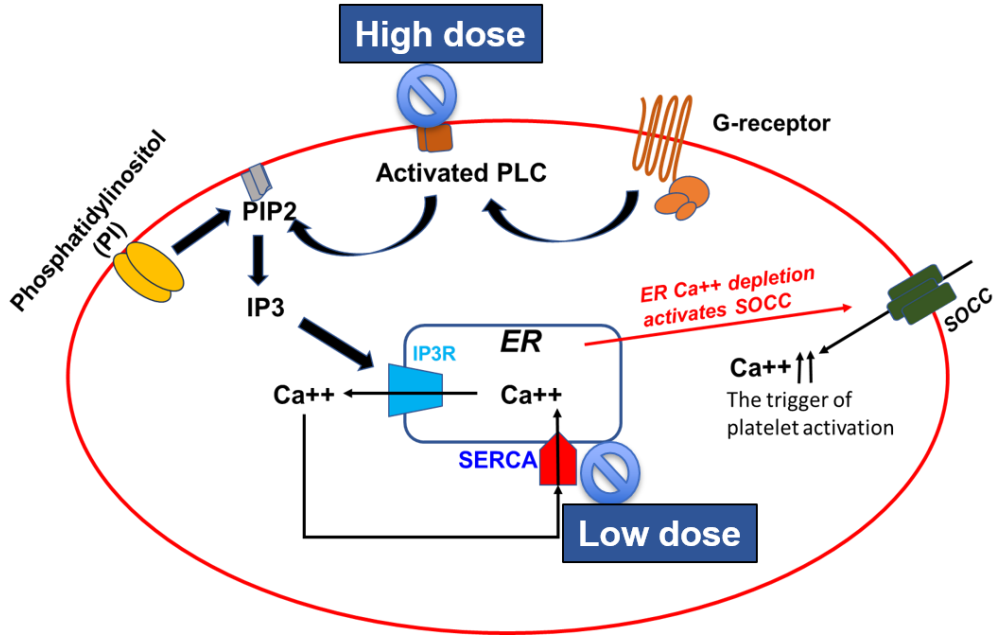
Does blocking calcium leak slow down metabolism in CT stored platelets?



We used different concentrations of U73122 (10, 20, and 50 μM) to control ER calcium leak.

Inhibition effect of U73122:

- Low dose: SERCA → Calcium leak
- High does: PLC and SERCA → inhibit calcium leak



Inhibited calcium leak and glucose consumption during CT storage