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Lipidomic changes occurring in platelets during extended cold storage

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Abstract

Objectives: Cold storage is being implemented as an alternative to conventional room-temperature storage for extending the shelf-life of platelet components beyond 5–7 days. The aim of this study was to characterise the lipid profile of platelets stored under standard room-temperature or cold (refrigerated) conditions.

Methods: Matched apheresis derived platelet components in 60% PAS-E/40% plasma ($n = 8$) were stored at room-temperature (20–24°C with agitation) or in the cold (2–6°C without agitation). Platelets were sampled on day 1, 5 and 14. The lipidome was assessed by ultra-pressure liquid chromatography ion mobility quadrupole time of flight mass spectrometry (UPLC IMS QToF). Changes in bioactive lipid mediators were measured by ELISA.

Results: The total phospholipid and sphingolipid content of the platelets and supernatant were 44 544 ± 2915 μg/mL and 38 990 ± 10 880 μg/mL, respectively, and was similar over 14 days, regardless of storage temperature. The proportion of the procoagulant lipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), increased by 2.7% and 12.2%, respectively, during extended cold storage. Cold storage for 14 days increased sphingomyelin (SM) by 4.1% and decreased ceramide by 1.6% compared to day 1. Further, lysophosphatidylcholine (LPC) species remained unchanged during cold storage for 14 days. The concentration of 12- and 15-hydroxyeicosatetraenoic acid (HETE) were lower in the supernatant of cold-stored platelets than room-temperature controls stored for 14 days.

Conclusion: The lipid profile of platelets was relatively unchanged during storage for 5 days, regardless of temperature. However, during extended cold storage (14 days) the proportion of the procoagulant lipids, PS and PE, increased, while LPC and bioactive lipids were stable.

KEYWORDS
lipid, platelet, transfusion

1 | INTRODUCTION

Conventionally, platelet concentrates are stored at room-temperature (RT) with constant agitation for up to 7 days.[1] The shelf-life is imposed in an effort to reduce the risk of bacterial proliferation and the effects of the platelet storage lesion (PSL).[2] Despite this, platelets still undergo a degree of storage associated activation. Current storage conditions also present issues for maintaining the supply of platelets in remote locations.

There is renewed interest in cold-stored (refrigeration at 2–6°C) platelets, as the increased shelf-life and improved haemostatic potential afforded by cold storage may outweigh the reduction in circulation time,[3,4] particularly if reserved for the treatment of active bleeding.[5] Cold-stored platelet components can currently be stored for up to 14 days due to the reduced risk of bacterial proliferation and reduction in metabolic rate.[6–10] During this time, cold storage induces changes to the membrane, including glycoprotein clustering, extracellular vesicle (EV) formation and phosphatidylserine (PS) externalisation.[11–13] Despite a growing body of evidence describing the impact of cold storage on platelet components, only a few historical studies have examined the lipidome.[14,15]

Previous lipidomic studies have examined platelet components stored in 100% plasma at both RT and cold temperatures.[14–18] However, it is necessary to provide an updated perspective that reflects current transfusion practices, including the use of platelet additive solution and storage beyond 7 days. Storage for 5–7 days at RT in 100% plasma resulted in a decrease in the total platelet lipid content and altered the overall composition of phospholipid, sphingolipid and cholesterol classes.[16,17] These lipid classes are the major constituents of the platelet lipid membrane and are the substrates for the formation of many bioactive lipid mediators, including lysophosphatidylcholine (LPC), ceramide (Cer), sphingosine 1-phosphate (S1P) and arachidonic acid derived lipid eicosanoids (5-hydroxyeicosatetraenoic acid (HETE), 12-HETE and 15-HETE).[19,20] As such, an assessment of the lipidomic changes resulting from cold storage was undertaken with a specific focus on the phospholipid and sphingolipid profile and their derived bioactive lipid mediators.

2 | METHODS

Ethics approval was obtained from the Australian Red Cross Lifeblood Research Ethics Committee prior to the commencement of this study. All donations were obtained from eligible, voluntary, non-remunerated blood donors.

2.1 | Preparation of platelet concentrates

Double-dose, leukoreduced apheresis platelet components ($n = 8$) were collected using a Trima Accel apheresis system (TerumoBCT, Lakewood, CO, USA), and stored in 40% plasma and 60% PAS-E (SSP +, Macopharma, Tourcoing, France). The platelet components were split into single dose equivalents, and the matched components were randomly assigned for storage at either RT (20–24°C) with constant agitation (Helmer Inc. Noblesville, IN, USA) or refrigerated (2–6°C) without agitation. The platelet components were aseptically sampled on day 1 (prior to allocation to storage temperature), 5 and 14 post collection, by docking on a sample pouch with a sterile-welding device. Prior to sampling, the cold-stored components were placed on an agitator at RT for 10 min to ensure that any platelets that had settled during storage were homogenously mixed.

2.2 | Analysis of platelet concentrates

The platelet count was determined using a haematology analyser (CELL DYN Ruby, Abbott Laboratories, Chicago, IL, USA). All platelet components were assessed for the presence of swirl and macroaggregates by visual inspection.[13]

The externalisation of phosphatidylserine was assessed by staining platelets with annexin-V and the percentage of positive cells were determined by flow cytometry (FACSCanto II, Becton Dickson, Franklin Lakes, NJ, USA).[11]

2.3 | Preparation of platelet and supernatant fractions

A standard number of platelets (500×10^6 platelets) were transferred to a 1.5 mL tube and centrifuged (Eppendorf 5415D; Eppendorf, Germany) at $1500 \times g$ for 15 min at RT to isolate the platelets. The supernatant was transferred to a new tube and the platelet pellet was stored at −80°C until analysis. The supernatant was then subjected to multiple, sequential rounds of centrifugation ($1500 \times g$ for 15 min at RT, $14000 \times g$ for 2 min at 4°C and $16\,000 \times g$ for 30 min at 4°C), with the supernatant being transferred to a new 1.5 mL tube between spins. The final supernatant was transferred to a new 1.5 mL tube and frozen and stored at −80°C until analysis.

2.4 | Lipid extraction and mass spectrometry

Lipids were extracted by methods adapted from Matyash et al.[21] A detailed description is presented in Supplementary Materials 1, but briefly, lipids were extracted from platelets (500×10^6) and supernatant (50 µL) by resuspension in methanol and addition of methyl tert-butyl ether (MTBE). Phase separation was induced by the addition of ddH_2O and the upper organic phase, containing lipids, was transferred to clean glass auto sampler vials. Global lipidomic analysis was conducted using an ACQUITY™ ultra-pressure liquid chromatography™(UPLC) I-Class system (Waters Corporation, Milford, MA, USA) coupled to a Vion Ion Mobility Spectrometry (IMS) Quadrupole Time-of-Flight (QToF) Mass Spectrometer (MS) (Waters Corporation, Milford, MA, USA) in an untargeted data-independent acquisition (DIA) manner. A detailed description of the chromatographic conditions and UPLC IMS QToF settings are presented in Supplementary Materials 1. Mass chromatograms were deconvoluted and data was normalised

using Progenesis QI software version 2.3 (Nonlinear Dynamics, a Waters Company, Newcastle upon Tyne, UK), according to manufacturer's workflow. Lipids were reported as normalised relative abundance, determined by the comparison of ion intensities of the internal standard, EquiSPLASH LIPIDOMIX Quantitative Mass Spectrometry Internal Standard (Avanti Polar Lipids, Alabaster, AL, USA), with a known concentration of lipid (μg/mL), or normalised to sum of ion content within a respective class (percentage %). Data are presented at the lipid species level as described by Liebisch et al.[22]

2.5 | Bioinformatics methodology for pathway analysis

Lipid pathway analysis was performed using the open access web-based tool, Bioinformatics Methodology For Pathway Analysis (BioPAN).[23] LC–MS/MS data was loaded into the platform and analysed according to the developer's workflow. BioPAN calculates a Z-score and determines if a given reaction is significant ($p < 0.05$). Further, a reaction is determined to be activated ($Z > 1.645$) or suppressed ($Z < -1.645$) depending on the direction of the change. The following options were selected; type: lipid, status: active, level: lipid subclass, subset of lipid data: reactions, p-value: 0.05, paired data: yes.

2.6 | Enzyme linked immunosorbent assays

The concentration of arachidonic acid (LSBio, Seattle, WA, USA), 5-HETE (LSBio), 12(S)-HETE (Abcam, Cambridge, UK), 15(S)-HETE (Abcam) and S1P (Echelon Bioscience, Inc., Salt Lake City, UT, USA) in the supernatant was determined using commercially available enzyme linked immunosorbent assay (ELISA) kits. All samples were tested in duplicate with the concentration determined from a standard curve.

2.7 | Statistical analysis

Results are expressed as mean ± standard deviation (SD). Data were analysed using GraphPad Prism (GraphPad Software Inc., Version 9, La Jolla, CA, USA). The effect of storage was assessed using a two-way repeated measures analysis of variance (ANOVA). Post hoc Bonferroni's multiple comparisons tests were performed to determine the differences between storage temperature (RT vs. cold-stored at day 5 and day 14) and time (day 1 vs. day 5 and day 1 vs. day 14). A p-value of less than 0.05 was considered statistically significant.

3 | RESULTS

The mean platelet concentration on day 1 was $1052 \pm 117 \times 10^9$/L. Although the platelet concentration decreased slightly during 14 days of storage to $953 \pm 123 \times 10^9$/L and $966 \pm 93 \times 10^9$/L under RT and cold storage conditions, respectively, there was no significant difference between the storage conditions ($p = 0.99$). Platelet swirl was apparent in all RT-stored components but not in cold-stored components. Macroscopic aggregates were not observed in any component.

3.1 | Characterisation of the phospholipid and sphingolipid profile of platelets

The effect of storage time and temperature on the lipid profile of platelets were assessed by UPLC IMS QToF. The total phospholipid and sphingolipid content of the day 1 platelets was 44 544 ± 2915 μg/mL and was similar over 14 days, regardless of whether the platelet components were stored at RT (48 101 ± 9704 μg/mL) or in the cold (44 405 ± 3908 μg/mL, $p = 0.99$).

Phosphatidylcholine (PC) is a major constituent of platelet lipid membranes and is the precursor for the bioactive lipid mediator, LPC, which has been associated with adverse transfusion reactions.[24–26] Compared to day 1, the percentage of PC was significantly lower in platelets after 14 days of cold storage, while the percentage of LPC was significantly lower in RT platelets at the same time point (Figure 1A). There were some shifts in PC species profile (Figure 1B), primarily at day 14 of cold storage. PC(34:2) and PC(36:3) were significantly higher in cold-stored components at day 14 compared to day 1, and compared to RT platelets at the same time point. In contrast, PC(36:1), PC(38:1) and PC(38:2) were significantly lower after 14 days of cold storage, compared to day 1. Only PC(38:3) increased during RT storage, at day 14. The LPC species profile was relatively stable during cold storage, with the exception of LPC(18:0), which was decreased at day 14, compared to day 1 (Figure 1C). In contrast, storage at RT resulted in a decrease in LPC(18:0), LPC(18:2), LPC(20:4) and LPC(22:4) by day 14. Conversely, LPC(20:0) and LPC(O-20:0) increased during RT storage.

The aminophospholipids, phosphatidylethanolamine (PE) and PS are structural membrane lipids that become externalised on the membrane surface to mediate coagulation, but also act a substrates for bioactive mediators and signalling molecules.[27] A significant increase (~4-fold; $p = 0.0051$) in the percentage of annexin-V positive platelets was observed at day 5 in the cold-stored platelets, indicative of increased phosphatidylserine externalisation (Figure 2A). However, by day 14 approximately 20% of platelets were annexin-V positive in both the cold and RT groups. Overall, the PS and PE lipid classes were significantly increased by cold storage at day 14, compared to day 1 (Figure 2B). In contrast, PEP was significantly decreased in platelets after 14 days of cold storage ($p = 0.0001$). In terms of specific species shifts, the most dramatic change was seen with PS(38:3), which increased significantly during cold storage, with a concomitant reduction during RT storage (Figure 2C). The proportion of PS(38:1) increased during storage in both groups, but the increase was greater during RT storage. Conversely, a time-related reduction in PS(40:1) and PS(40:3) was observed, to a similar degree in both the cold and RT platelets. Multiple differences were found in the PE species as a result of storage time and temperature (Figure 2D). In general, PE (34:2), PE(36:1), PE(36:2) and PE(40:4) increased during storage, but to a significantly higher extent in cold-stored components at day 14.

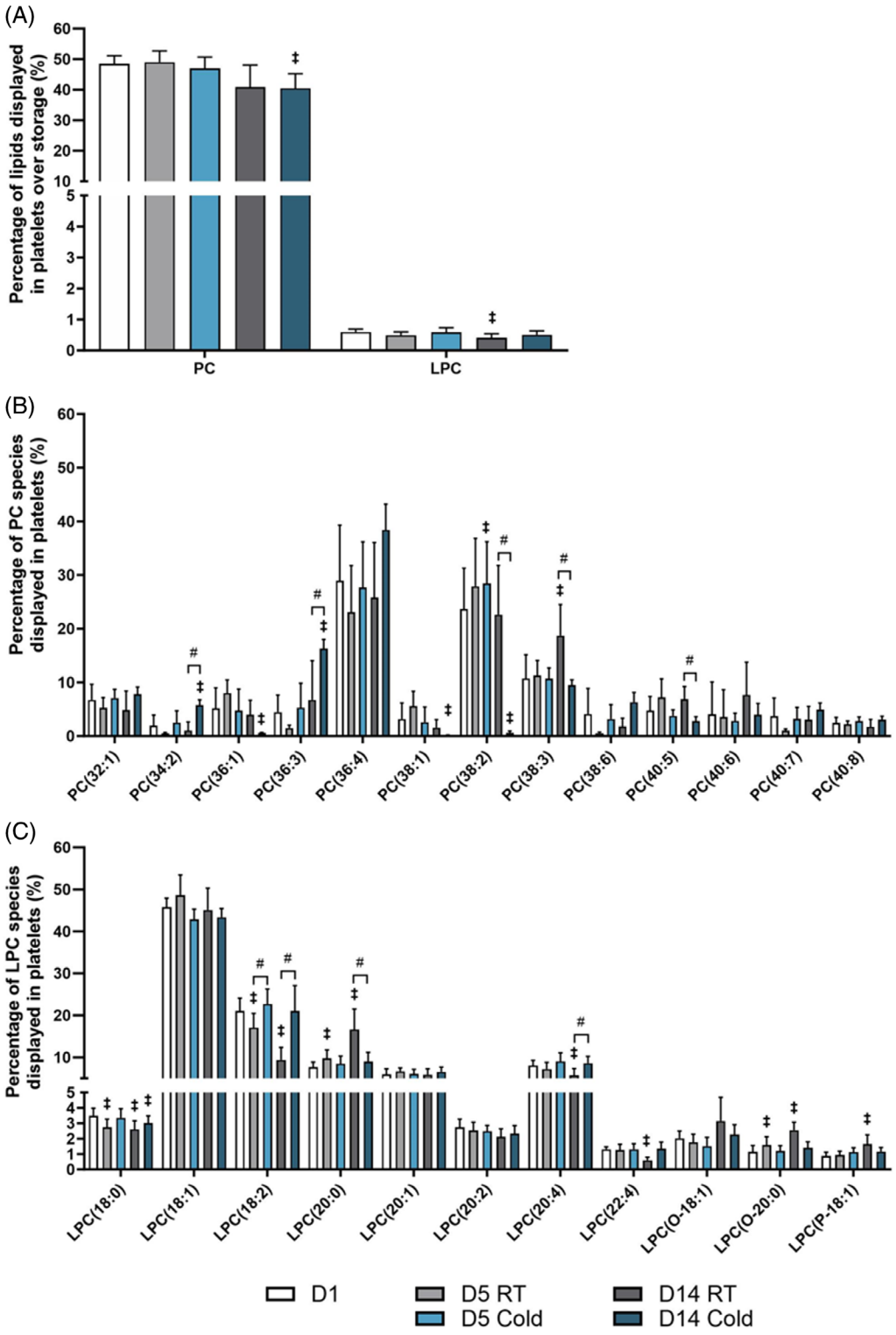

FIGURE 1 The effect of cold storage on phosphatidylcholine and lysophosphatidylcholine. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (A) phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) classes and (B) PC and (C) LPC lipid species are displayed. The data represents mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

A time related increase in PE(38:4) was observed, with both temperature groups showing a significant increase at day 14, compared to day 1. PE(38:1) and PE(40:2) showed the opposite trend, with significant reductions observed at day 14, compared to day 1. In terms of PEP species, PE(P-40:1) represented the most abundant species, and only minor shifts in low abundance species were observed (Figure 2E).

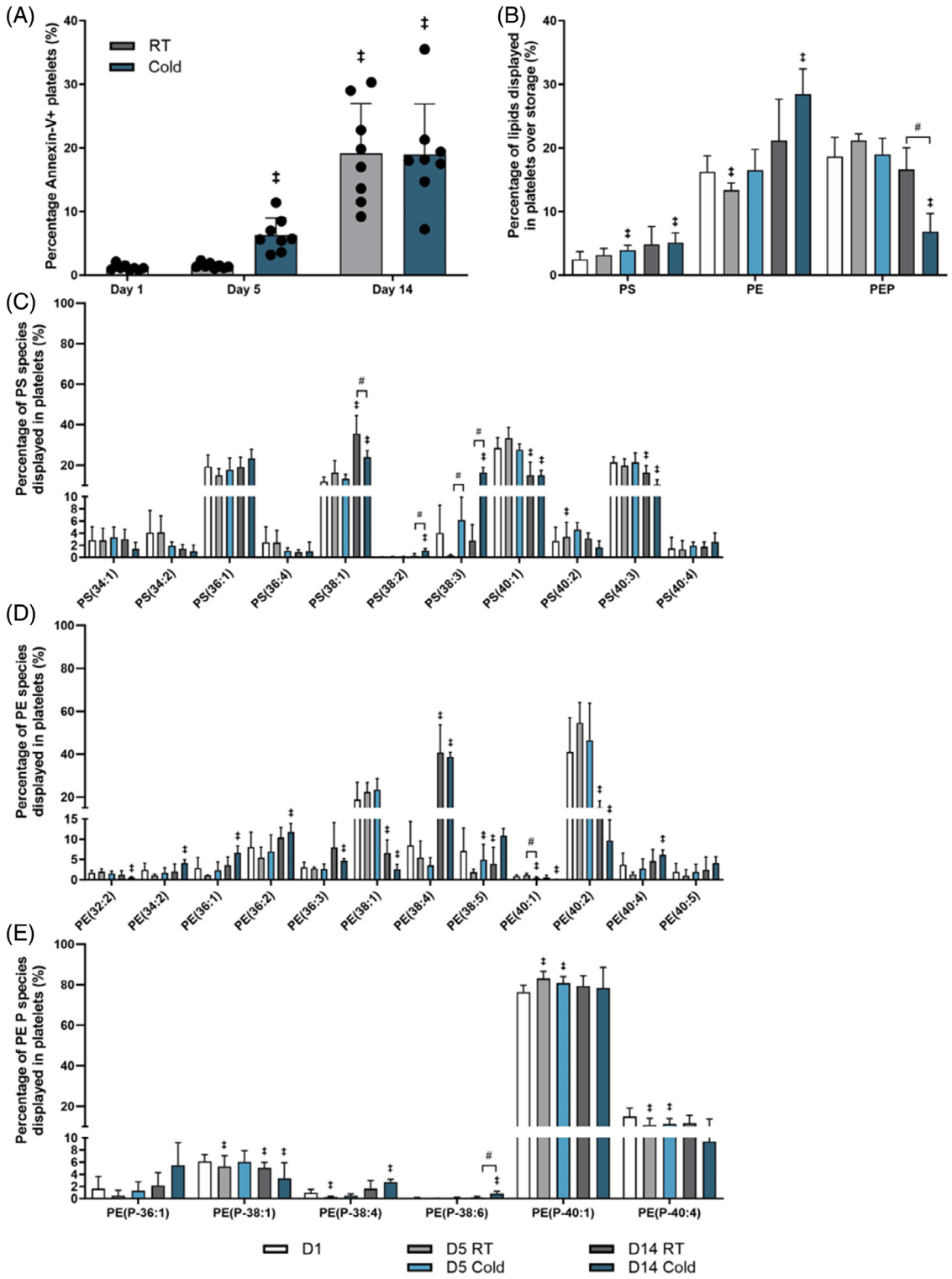

FIGURE 2 The effect of cold storage on phosphatidylserine, phosphatidylethanolamine and ether linked phosphatidylethanolamine. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). (A) Phosphatidylserine (PS) externalisation was measured by flow cytometric analysis of annexin-V (FITC) positive platelets. The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (B) PS, phosphatidylethanolamine (PE) and ether linked phosphatidylethanolamine (PEP) and lipid species of (C) PS, (D) PE and (E) PEP are displayed. The data represents individual data points, mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

Sphingomyelin (SM) is a precursor to the second messengers and bioactive lipid mediators, ceramide, sphingosine and S1P.[28] SM was significantly increased during cold storage, compared to day 1, and to RT-stored platelets at day 14 (Figure 3A). In contrast, Cer was significantly decreased in cold-stored platelets after 14 days ($p = 0.0016$), while being significantly higher in platelets stored at RT. The

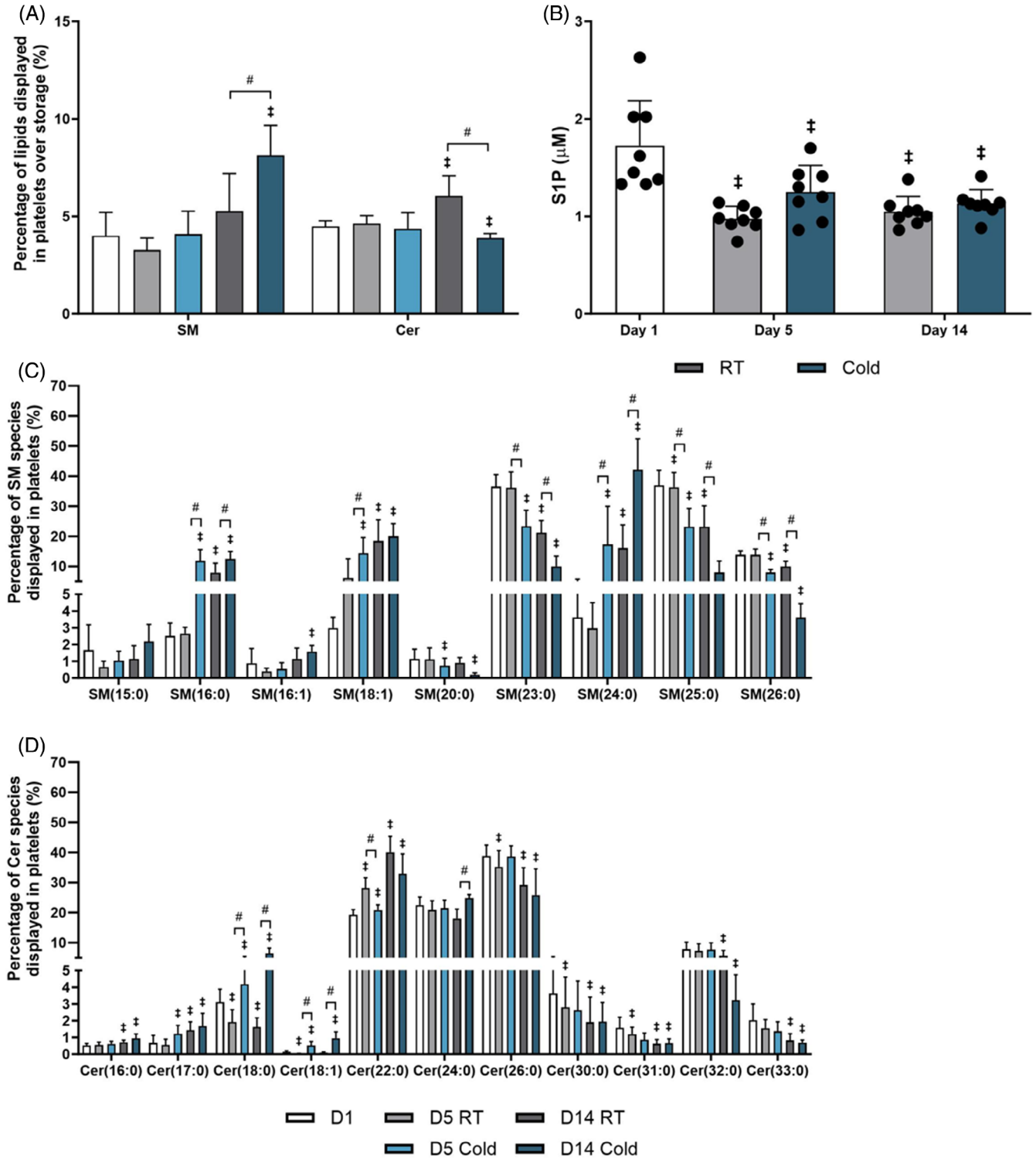

FIGURE 3 The effect of cold storage on sphingomyelin and ceramide. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The (A) percentage composition of sphingomyelin (SM) and ceramide (Cer) are displayed. The concentration of (B) sphingosine 1-phosphate (S1P) was determined by ELISA. The percentage composition of (C) SM and (D) Cer lipid species are displayed. The data represents mean (bars) + SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

concentration of S1P decreased over storage, but was similar between the storage temperatures (Figure 3B). In terms of species changes, SM(16:0), SM(18:1) and SM(24:0) increased during storage, and cold storage amplified this effect (Figure 3C). Conversely, the opposite effect was seen with SM(23:0), SM(25:0) and SM(26:0), which decreased during storage at both temperatures, with a greater

loss of these species in cold-stored platelets. Regarding ceramide species, there was a trend for short chain ceramide species to increase over storage (Figure 3D). More specifically, Cer(18:0) and Cer(18:1) increased in cold-stored samples, with a concomitant decrease in RT-stored platelets. Cer(22:0) was significantly increased at day 5 and day 14 in both groups, and the increase was more pronounced in RT stored samples. Cer(24:0) was stable during early storage, but a significant difference between RT and cold platelets was observed after 14 days of storage. Interestingly, the long chain ceramide species tended to decrease with storage time regardless of temperature.

3.2 | Assessment of lipid dynamics and lipid pathways

The metabolic pathways involved in converting the lipids in platelets are regulated by well characterised enzymatic catalytic conversions.[18,19] As an integral part of platelet function, it is not only important to understand quantitative changes but also to understand how the metabolic pathways are affected. BioPAN is an open access web-based tool that provides a statistical score for possible lipid metabolism pathways.[23] Lipid networks were computed, comparing the storage time (day 1 vs. day 5 and day 5 vs. day 14), for RT and cold-stored

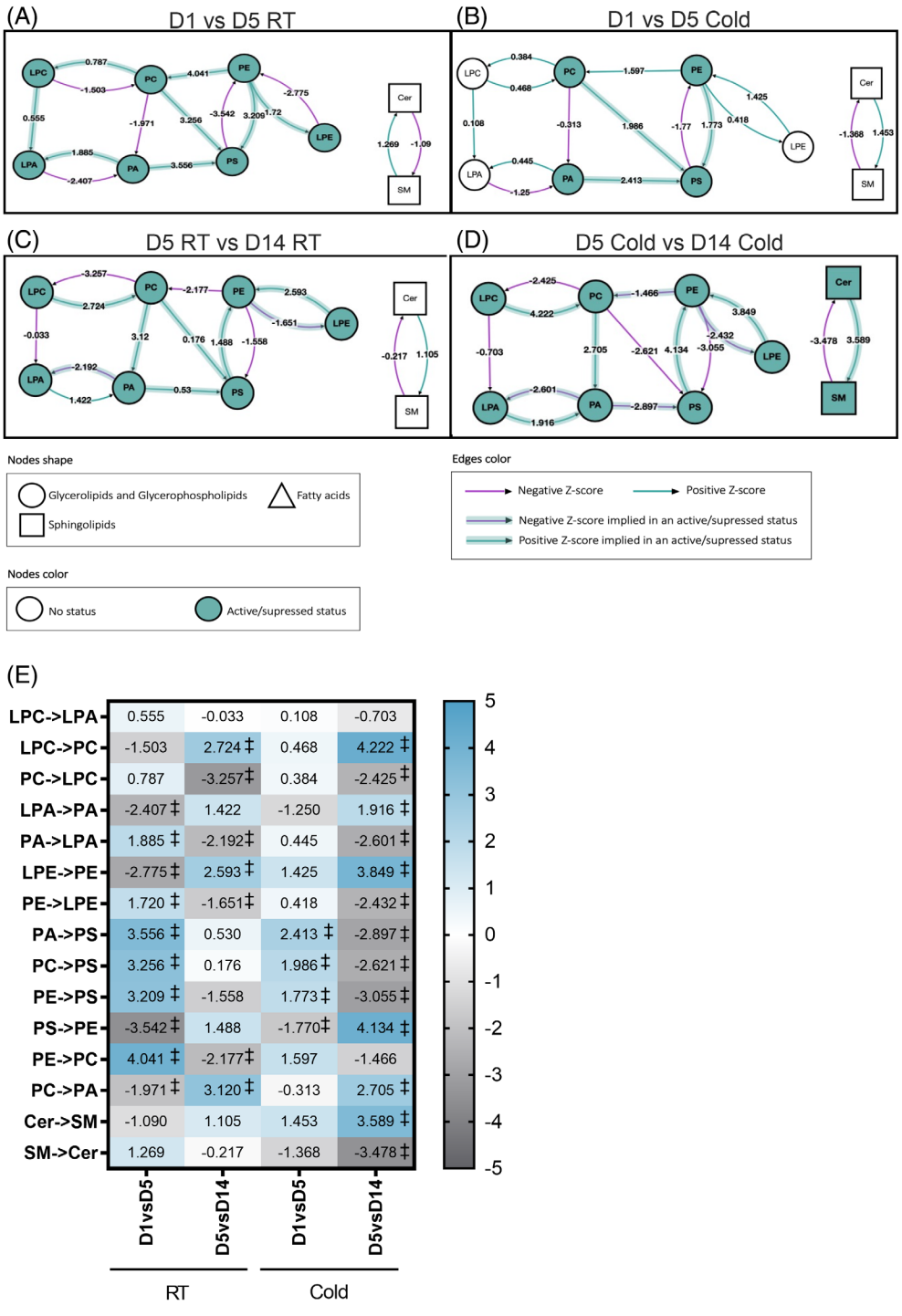

FIGURE 4 The effect of cold storage on lipid metabolism as predicted using BioPAN. Lipid networks were generated from BioPAN using data acquired by UPLC IMS QToF. The lipid networks comparing samples at day 1 (D1) with (A) day 5 room temperature (D5 RT) or (B) day 5 cold (D5 Cold) and (C) room temperature samples at day 5 (D5 RT) versus day 14 (D14 RT) or (D) cold samples at day 5 (D5 Cold) versus day 14 (D14 Cold). (E) The Z-scores from the lipid network summarised and depicted as a heat map. Values shown as Z-score of the given reaction, whereby a Z-score >0 represents an active reaction (blue) and a Z-score <0 represents a suppressed reaction (grey). A paired t-test was performed by BioPAN to determine differences between indicated groups. ‡ indicates $p < 0.05$ a significant reaction (corresponding to a Z-score >1.645 or <−1.645).

platelets (Figure 4A–D). The BioPan lipid network showed distinct differences in lipid pathways depending on storage conditions, as summarised in the Heat Map (Figure 4E). During RT storage, pathway activity was greater between day 1 and day 5. Conversely, during cold storage the greatest activity in lipid pathways occurred between day 5 and day 14. PS formation by phosphatidic acid (PA), PC, and PE was downregulated by cold storage between day 5 and day 14, while the catabolism of PS to PE was an active pathway during prolonged cold storage. Further, cold storage drastically altered sphingolipid metabolism. Specifically, between day 5 and day 14 of cold storage the formation of SM from Cer was a highly active pathway.

3.3 | Characterisation of the phospholipid and sphingolipid profile of the supernatant

Recipients of platelet transfusions will receive both the platelets and the contents of the storage solution (plasma, platelet additive solution, biological response modifiers and EVs). The lipidome of platelets and the platelet component is dynamic and lipids are released or exchanged with the surrounding environment, or transformed by enzymatic reactions.[20] Thus it is important to consider the storage solution and its contribution to the overall lipid profile of the platelet component. The phospholipid and sphingolipid content of the supernatant fraction was assessed by UPLC IMS QToF. The mean concentration on day 1 was 38 990 ± 10 880 μg/mL, which remained similar over 14 days, regardless of whether the platelet components were stored at RT (33 541 ± 5661 μg/mL) or in the cold (36 727 ± 10 880 μg/mL, $p = 0.99$ when comparing RT and cold-stored at day 14).

In terms of lipid classes, numerous changes were apparent in the supernatant. Lipid changes in the supernatant were observed at day 5 when platelet components were stored at RT (Figure 5A), while lipids in the supernatant of cold-stored platelets components were relatively stable throughout 14 days of storage. There was a decrease in PC, PEP and Cer in both RT and cold storage, compared to day 1, with a trend for a larger decrease in the RT group at day 5. Conversely, lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), PE, phosphatidylinositol (PI) and PS were significantly increased during RT storage, compared to day 1. Similarly, PI and PS were significantly increased during cold storage, compared to day 1. Despite these changes, only small and subtle changes were observed in the lipid species of most classes (PC, PS, PE, PEP SM and Cer) (Supplementary Materials 2; Figures S1, S2 and S3).

While the overall proportion of the LPC class did not differ as a result of storage time or temperature, differences at the species level were of interest due to their association with transfusion outcomes.[26] LPC(18:1) increased during storage at both temperatures, although the increase was greater during RT storage. Concomitantly, LPC(18:2) decreased over storage at RT (Figure 5B). Overall, with the exception of LPC(18:1), the LPC species profile was relatively unchanged by cold storage until day 14.

The concentration of several bioactive lipid mediators in the supernatant was measured by ELISA. The concentration of arachidonic acid and 5-HETE remained relatively constant over storage, regardless of storage temperature (Figure 5C,D). However, the concentration of 12(S)-HETE and 15(S)-HETE increased during storage, with a larger increase during RT storage (Figure 5E,F).

4 | DISCUSSION

A paucity of recent data examining the lipidome of cold-stored platelets exists, particularly regarding extended storage in platelet additive solution. This study assessed the lipidomic changes occurring in platelets stored for the current (5–7 days) and feasible (14 days) shelf-life of RT and refrigerated components, respectively. The overall phospholipid and sphingolipid profile of platelets stored in additive solution at day 1 was similar to that previously reported for platelets in plasma,[16,17] and cold storage had little impact during early storage (5 days). However, the proportion of PE, PS, and SM classes increased during extended (14 days) cold storage, while PC, PEP and Cer decreased, compared to day 1.

Differences in phospholipid content between RT and cold-stored platelets may arise due to altered lipid metabolism or a change in the kinetics of incorporation from plasma lipoproteins; both of which are temperature dependent.[29] Further, supplementation with platelet additive solution, which is now standard practice in many blood collection centres, may reduce the concentration of plasma enzymes responsible for much of the lipid metabolism occurring during platelet storage.[18] Thus, differences between the changes in the lipid profile over storage between this and other studies[14–17] are likely due to differences in the storage solution.

The externalisation of aminophospholipids, PS and PE, is essential to support normal haemostatic function.[27] Further, long chain fatty acid species, such as arachidonic acid (20:4), provide better support for coagulation compared to short chain species.[30] While there was no change in arachidonic acid containing PS species, an increase in PE and PEP species, in which one of their fatty acid tails could be arachidonic acid (20:4), such as PE(38:4), PE(40:4) and PE(P-38:4) was observed. These subtle shifts may contribute to the increased haemostatic profile of cold-stored platelets.[13,31]

Recently cold storage has been shown to alter the immunomodulatory potential of platelets, evidenced by changes in the abundance of toll-like receptors on the platelet surface and release of immunomodulatory bioactive response modifiers during storage.[32] While certain LPC species, including 16:0, 18:0, 18:1, 18:2, 20:4 and 22:0, have been associated with inflammatory processes,[33,34] their exact roles remain unclear due to limited investigations. Further, transfusion of components containing high concentrations of polar (LPC and LPC derivatives) and non-polar (12-HETE and 15-HETE) bioactive lipid mediators are associated with TRALI.[24–26,35] During RT storage, LPC (18:1) increased, while LPC(18:2) decreased in the supernatant, which may have opposing effects. Similarly, the concentration of 12-HETE and 15-HETE increased to a greater degree in platelets stored at RT. Overall, cold storage resulted in less accumulation of these mediators, which may be a result of the temperature dependence of enzymatic reactions, decreased metabolic activity of the platelets and lower granule release.[13,36,37] Taken together, cold storage may be beneficial in reducing the incidence of transfusion reactions.

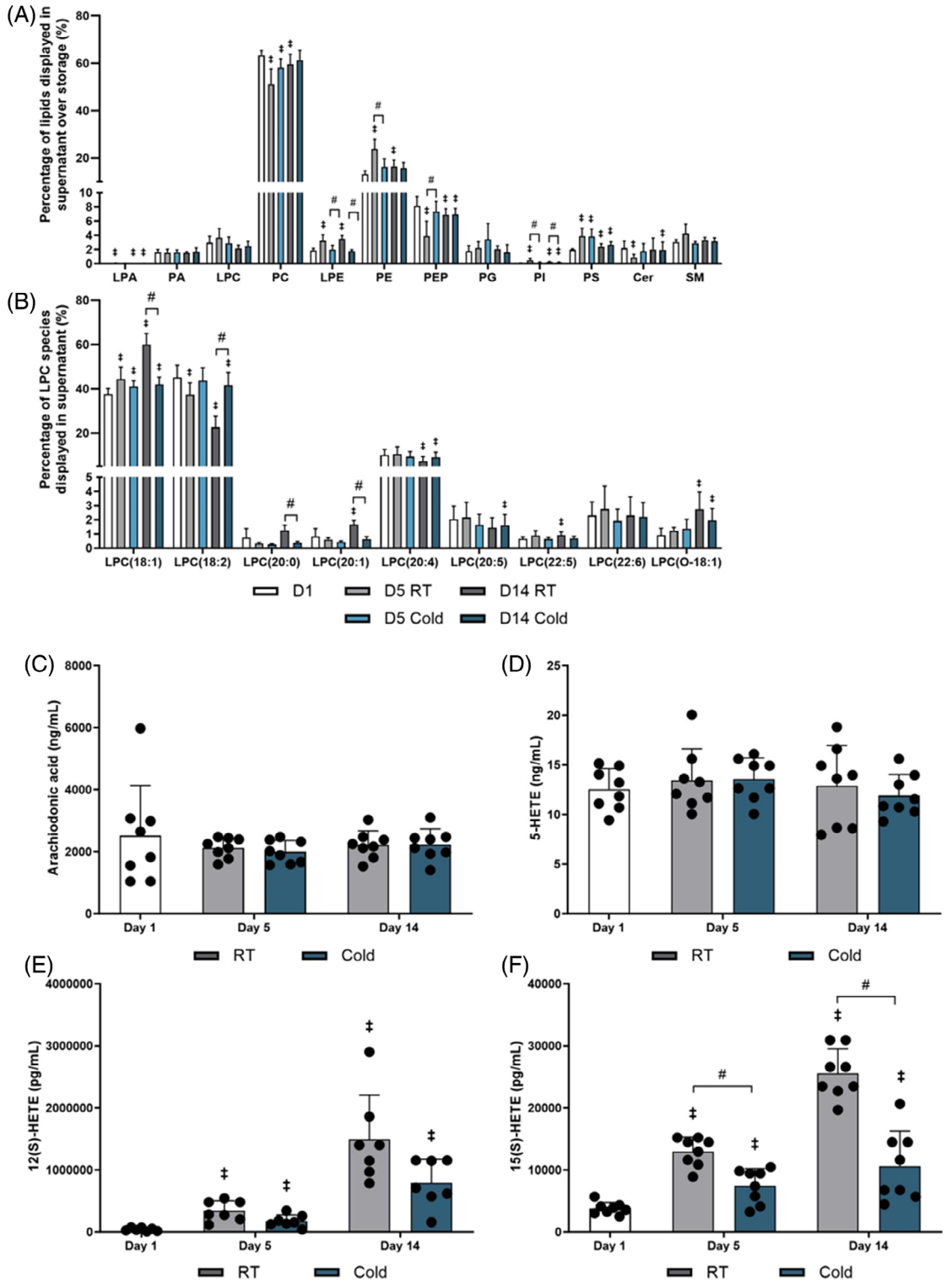

FIGURE 5 The effect of cold storage on the supernatant lipid profile. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The supernatant was isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (A) lipid classes and (B) lysophosphatidylcholine (LPC) species in the supernatant are displayed. The concentration of (C) arachidonic acid, (D) 5- hydroxyeicosatetraenoic acid (HETE), (E) 12(S)-HETE and (F) 15(S)-HETE were determined by ELISA. The data represents individual data points, and mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

Sphingolipid metabolism is unique in platelets as they lack the enzymes necessary for de novo synthesis and S1P catabolism.[38] The catabolism of SM is considered to be an essential pathway by which potent bioactive lipid mediators, such as Cer and S1P, are formed.[38] Pienimaeki-Roemer et al., report that during conventional RT storage, S1P catabolism results in ceramide formation though the

transmembrane cycling and salvage pathway.[16,39] In this study, RT stored platelets followed a similar trend, where decreased S1P was associated with an increase in Cer, although no change in SM was observed. In contrast, while a decrease in S1P was also observed in cold-stored platelets, this was associated with a significant decrease in Cer, while SM significantly increased. Taken together, these results suggest that platelet storage temperature influences sphingolipid metabolism.

Ceramide has received considerable attention as a key regulator of apoptosis in nucleated cells,[40,41] although this has not been investigated in platelets. The elevated ceramide levels and low S1P observed in platelets stored at RT for 14 days suggests that the sphingolipid rheostat is tipped towards a cell death fate. This aligns with previous in vitro data, whereby RT platelets exhibit increased apoptotic signalling during prolonged storage (beyond 7 days), while the progression of apoptosis is delayed by cold storage.[7,42] It remains to be determined whether specific ceramide species (e.g., Cer 16:0, Cer 18:0 and Cer 22:0) are involved in mediating apoptosis in platelets, as they have been implicated in other cell types.[40,41] This is of particular interest as many ceramide species were found to be differentially modulated by RT and cold storage.

The differential modulation of ceramide in stored platelets may also have implications for EVs, as ceramide promotes EV formation.[43] Previous studies have shown that cold-stored platelets release more EVs into the supernatant,[13,36,44] which is interesting given that this study shows ceramide did not increase in cold-stored platelets over storage. It is speculated that perhaps the apparent lack of ceramide accumulation in cold-stored platelets may arise as a result of the platelet ceramide being packaged into EVs. Targeted investigations of the lipid content of EVs in stored platelets are required to specifically address this.

Opportunities for further study arise from the limitations of our study. Our data was obtained from a relatively small number of apheresis donors ($n = 8$), so donor variability may limit the generalisability of the findings.[45] Further, linking the identified lipid changes to the functional capacity of the platelets, as has recently been done with metabolomics,[46] could improve our understanding of certain aspects of platelet biology and storage. As mentioned above, while the lipid content of EVs generated during cold storage was not interrogated in this study, this information would be valuable given that EVs can serve as shuttles, transferring their lipid content to other cells, to mediate inflammatory processes such as lung injury.[16,43]

In summary, this work provides novel insight into changes in the lipid profile of platelet components during extended storage at both RT and cold storage. We demonstrate that the sphingolipid and phospholipid profile of platelets and the supernatant were differentially altered by storage time and temperature. It is apparent that storage for 5 days does not drastically affect the lipid profile. However, as storage progresses, differences in lipid classes and species associated with coagulation, apoptosis and inflammation become apparent. These changes may have functional effects once the components are transfused, warranting further studies to understand the clinical consequences of these changes.

AUTHOR CONTRIBUTIONS

Lacey Johnson, Matthew P. Padula, and Denese C. Marks designed the study. Tyren M. Dodgen and Amani Batarseh provided intellectual input on experimental design. Sarah M. Green, Lacey Johnson and Matthew P. Padula performed the research. Sarah M. Green and Lacey Johnson analysed the data, prepared the figures, and drafted the manuscript. All authors contributed to writing, critically revised, and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Reprints will not be made available from the author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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