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ORIGINAL ARTICLE

Carbon dioxide alleviates platelet storage lesions via stimulating fatty acid metabolism and reducing platelet glucose consumption

Shunli Gu | Jinmei Xu | Erxiong Liu | Xuejia Hou | Ning An | Yaozhen Chen | Zhixin Liu | Wenting Wang | Xingbin Hu | Wen Yin

Department of Transfusion Medicine, Xijing Hospital, The Fourth Military Medical University, Xi'an, China

Correspondence
Xingbin Hu and Wen Yin, Department of Transfusion Medicine, Xijing Hospital, The Fourth Military Medical University, Changle Rd., Xi'an 710032, China.
Email: hxbyqh@fmmu.edu.cn and yinwen@fmmu.edu.cn

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Abstract

Background: The timely administration of platelet transfusions is critical for patient survival, and the clinical demand for platelet transfusions has been steadily increasing. However, platelet storage lesions (PSLs) that develop during *in vitro* preservation exacerbate these shortages. The PSL is significantly influenced by various factors, including temperature, gas composition, and buffering systems. Strategies to mitigate PSLs and improve platelet storage have been actively explored in recent years.

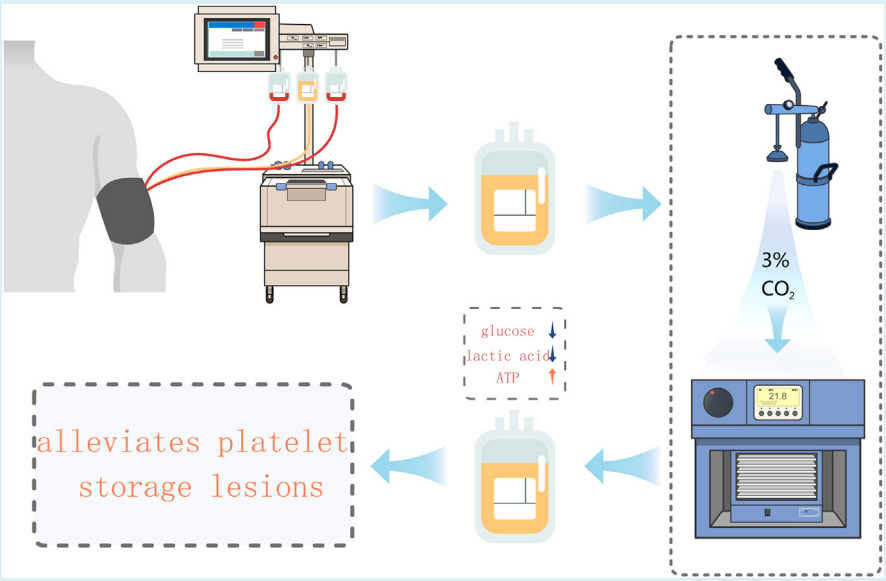
Objectives: This study aimed to investigate whether elevated carbon dioxide (CO_2) levels improve platelet quality and functionality during storage.

Methods: Platelet concentrates from 28 donors were stored under control or 3% CO_2 conditions at 22 ± 2 °C for up to 7 days. Platelet quality was evaluated through scanning electron microscopy, adhesion, aggregation, clot contraction, activation, apoptosis assays, blood gas, adenosine triphosphate, metabolomics analyses, and *in vivo* thrombosis and survival tests.

Results: Our findings indicate that increasing the CO_2 concentration in the storage environment mitigates PSLs and improves platelet quality.

Conclusion: Our study highlights the potential benefits of utilizing a high CO_2 storage environment to improve platelet preservation, offering a promising method to address clinical platelet shortages.

Shunli Gu and Jinmei Xu contributed equally to this study.



KEYWORDS
CO_2, metabolism, platelet, platelet transfusion, platelet storage

Essentials

- Platelet quality during storage often declines, causing shortages and limiting transfusion success.
- This study tested the effects of CO_2 on platelet quality during storage.
- We discovered that 3% CO_2 alleviates platelet storage lesions during storage.
- The findings underscore the potential of CO_2 to improve platelet quality during storage.

1 | INTRODUCTION

Platelets are a vital blood component, playing essential roles in coagulation, hemostasis, and wound healing [1]. These small cells initiate the hemostatic response, leading to vasoconstriction and platelet plaque formation [2]. Platelets contribute significantly to immune responses, including defense against infection and inflammation [3,4]. Consequently, platelet transfusions are widely utilized to treat and prevent bleeding [5].

Platelet storage lesions (PSLs) encompass morphologic [6] and functional changes that occur during preparation and storage, including platelet apoptosis, activation, reduced aggregation capacity, and mitochondrial dysfunction [7]. Temperature, gas, and buffer compositions significantly influence PSLs [8]. These lesions lead to reduced platelet quality, impaired hemostatic function, and diminished therapeutic efficacy of transfusions [9]. As platelet demand in clinical settings grows, reducing PSLs and developing innovative platelet storage techniques have become increasingly urgent.

Lactic acid accumulates in platelets during storage, causing morphologic alterations and eventual apoptosis, ultimately shortening their storage duration [10]. In the platelet storage environment, lactic acid primarily arises as a metabolic byproduct of glucose metabolism [11,12]. During *in vitro* storage in plasma, platelets primarily derive their adenosine triphosphate (ATP) from anaerobic respiration, which consumes significant amounts of glucose and produces lactic acid. Consequently, this process leads to lactic acid accumulation, culminating in a decline in pH, a major contributor to PSLs [8,10,13]. To mitigate lactic acid accumulation and maintain pH, platelets are currently stored in air-permeable bags with agitation, which enhances carbon dioxide (CO_2) and oxygen (O_2) exchange, maximizing aerobic respiration efficiency [14]. Thus, strategies targeting the reduction of lactic acid accumulation warrant continued exploration in platelet storage systems.

Studies have demonstrated that CO_2 can influence cell metabolism and lactic acid production [15,16]. As a nonpolar molecule, CO_2 can freely cross cell membranes, reducing intracellular pH and altering cellular metabolism [17]. Several studies have shown that in conditions of intestinal endotoxin-induced hypercapnia, CO_2 prevents ATP depletion and lactate accumulation [18–20]. However, the potential effects of CO_2 on platelet metabolism and lactate production during storage remain unexplored.

In this study, we hypothesized that elevated CO_2 concentrations would reduce PSLs.

To test this hypothesis, platelets were stored under high CO_2 conditions, and their properties and functions were monitored.

2 | METHODS

2.1 | Preparation and storage of apheresis-derived platelet concentrates

Following China's blood donation guidelines, the apheresis-derived platelet concentrates (APCs) were collected from 28 healthy volunteers using the Amicus 4R4580 (Fenwal) apheresis separator. Each volunteer donated 2 therapeutic doses of APCs (250 mL, 2.5×10^{11} platelets per dose). On day 0 (the day of APC collection), the APCs were divided into a control group and a 3% CO_2 group and stored at 22 ± 2 °C in a platelet incubator with gentle agitation for 3, 5, or 7 days. The control group was stored in a platelet incubator (PC900i, Helmer), while the 3% CO_2 group was stored in a 3-gas incubator (ALSM, Xi'an Beiguang Medical Biotechnology) under 3% CO_2 conditions. After storage, the platelets were diluted to an optimal concentration using platelet-poor plasma. The platelet count of the stored samples was determined using a hematology analyzer (XP-100, Sysmex). The Ethics Committee of Xijing Hospital approved the study protocol, and informed consent was obtained from all platelet donors.

2.2 | Scanning electron microscopy observation

The stored APCs (10 μL, 1×10^9/mL) were mixed with 0.99 mL of 2.5% glutaraldehyde solution (Sigma) and refrigerated at 2 to 6 °C for 12 hours. A 50 μL sample was then placed on a slide, centrifuged at 200 × *g* for 2 minutes, air-dried, rinsed with phosphate buffer saline (PBS), dehydrated with ethanol, and vacuum freeze-dried. Morphologic examination of platelets was performed using scanning electron microscopy (SEM; S-3400N, Hitachi) after gold labeling.

2.3 | Platelet adhesion assay

Before platelet introduction, slides were precoated with either fibrinogen (100 μg/mL, Sigma) or collagen (100 μg/mL, Sigma). Subsequently, the stored APCs (100 μL, 1×10^8/mL) were dispensed onto the precoated slides. The slides were then incubated at room temperature (RT) for 1 hour. Adherent platelets were washed thrice with PBS and fixed with 4% paraformaldehyde at RT for 20 minutes. Images were captured using a light microscope (Z30, Nikon).

2.4 | Platelet aggregation test

The stored APCs (140 μL, 0.4×10^9/mL) were evaluated using a hemagglutometer (CS-2400, Sysmex). The platelet aggregation rate was then measured using the Platelet Aggregation Function (ADP) Assay Kit and the Platelet Aggregation Function (AA) Assay Kit (Sysmex), following the manufacturer's instructions.

2.5 | Clot contraction analysis

The stored APCs (250 μL, 1×10^9/mL) were incubated at 37 °C with thrombin (0.5 U/mL, Sigma) and $CaCl_2$ (0.025 mol/L, Aladdin). Images were captured at 0 minutes (before incubation) and 10 and 20 minutes after incubation. Clot sizes were then analyzed using ImageJ software (version 1.8.0, National Institutes of Health).

2.6 | Animals and thrombosis test *in vivo*

Ten- to 12-week-old NOD-SCID mice were obtained from Cyagen and housed in a specific pathogen-free environment. The Animal Resources Centre of the Fourth Military Medical University reviewed and approved all animal protocols and ethical statements. An antimouse CD42b monoclonal antibody (0.5 μL/g, Emfret) was administered through the tail vein to deplete autologous platelets in the mice. Anesthesia was induced 24 hours later using 1.25% tribromoethanol (30 μL/mg, Nanjing Aibei Biotechnology). Following anesthesia, rhodamine 6G solution (0.5 mg/mL, 8 μL/g, Sigma) was administered intraperitoneally. Stored APCs (1×10^9/mL, 18 μL/g) were injected through the tail vein. The sublingual gland was separated, and the carotid artery was fully exposed. Filter paper soaked in $FeCl_3$ solution (200 mg/mL, Aladdin) was placed on the exposed carotid artery for 2 minutes. Carotid artery thrombosis formation was observed using a stereofluorescence microscope (AXIO Zoom.V16, ZEISS).

2.7 | Bleeding time calculation

Antimouse CD42b monoclonal antibody was administered through the tail vein to deplete autologous platelets in NOD-SCID mice. Stored APCs (1×10^9/mL, 18 μL/g) were injected through the caudal vein. The tip of the murine tail was amputated, and the bleeding time was recorded.

2.8 | Platelet survival rate evaluation *in vivo*

Stored APCs (1×10^9/mL, 14 μL/g) were injected into 12-week-old NOD-SCID mice via the tail vein. Subsequently, 50 μL of peripheral blood samples were collected at 0.5, 2, and 5 hours. The samples were

centrifuged at 250 × *g* for 10 minutes, and 1 mL of ACK Lysis Buffer (Boster) was added to the pellet to lyse red blood cells. After 1 minute, 2 mL of PBS was added, and the sample was centrifuged at 250 × *g* for 10 minutes. The pellet was then fixed with 4% paraformaldehyde for 5 minutes. Subsequently, 1 mL of flow cytometry staining buffer was added, and the sample was centrifuged at 250 × *g* for 10 minutes to wash. Finally, the platelets were labeled with APC-conjugated anti-human CD42a (BioLegend) and FITC-conjugated anti-mouse CD42a antibodies (BioLegend) and analyzed using flow cytometry (FACS Canto, BD).

2.9 | Platelet activation and apoptosis assay

Platelet activation was analyzed by assessing CD62P and CD42b using flow cytometry, while platelet factor 4 (PF4) was measured via enzyme-linked immunosorbent assay (ELISA). Specifically, stored APCs (1 μL, 1 × 10^9/mL) were washed and incubated with 5 μL FITC-conjugated antihuman CD62P (BD) and 5 μL APC-conjugated antihuman CD42b (BioLegend) for 15 minutes at RT before flow cytometry analysis. To determine PF4 concentration, 200 μL of supernatant from stored APCs (300 μL, 1 × 10^9/mL) was processed following the PF4 ELISA Kit instructions (Boster). Absorbance was measured using a microplate reader (Synergy LX, Biotek). Regarding apoptosis analysis, stored APCs (1 μL, 1 × 10^9/mL) were washed and incubated with 5 μL annexin V-FITC (BD) for 15 minutes following the instructions provided with the Apoptosis Detection Kit (BD). The cells were then analyzed using flow cytometry (FACS Canto, BD).

2.10 | Blood gas analysis

The glucose, lactic acid, CO_2 partial pressure, pH, O_2 partial pressure, and bicarbonate (HCO_3^-) concentrations were measured using blood gas analysis. Stored APCs (500 μL, 1 × 10^9/mL) were analyzed using a fully automated blood gas analyzer (GEM Premier 3500, Werfen).

2.11 | ATP concentration evaluation

The stored APCs (200 μL, 1 × 10^9/mL) were centrifuged at 1000 × *g* for 5 minutes to isolate the platelets. Following this, 500 μL of lysate buffer was added, and the mixture was centrifuged at 12,000 × *g* at 4 °C for 5 minutes. The supernatant was transferred to a new tube, and ATP concentration was measured according to the instructions provided with the ATP Assay Kit (Beyotime).

2.12 | Metabolomics analysis

Platelets stored for 3 days underwent metabolomics analysis. Washed APCs (1 mL, 1 × 10^9/mL) were isolated. Metabolomics analysis was conducted using high-performance liquid chromatography-tandem mass spectrometry with the analytical instrument LC-MS QTRAP 6500+ (SCIEX) at the Beijing Genomics Institution. Free fatty acid levels were also measured using a Free Fatty Acid Detection Kit (Jiuqiang Biotechnology) and a fully automated biochemical analyzer (AU5800, Beckman).

2.13 | p38 adenosine monophosphate-activated protein kinases phosphorylation detection

The stored APCs (500 μL, 1 × 10^9/mL) were collected and washed thrice with PBS to remove plasma. The platelets were lysed with RIPA Buffer (Boster) and denatured by boiling for 10 minutes in an SDS loading buffer (Thermo Fisher Scientific). Phosphorylation of p38 adenosine monophosphate-activated protein kinases (AMPK) in platelets was analyzed using monoclonal antibodies against human p38 AMPK (1:1000, Cell Signaling Technology) and phospho-p38 AMPK (1:1000, Cell Signaling Technology) by Western blot.

2.14 | Statistical analysis

All data were analyzed using GraphPad Prism 8.0. Differences were compared using paired *t*-tests. Multiple comparisons were conducted using a 2-way analysis of variance with Tukey's posttest. Data are presented as mean ± SEM. Statistical significance was defined as $P < .05$.

3 | RESULTS

3.1 | CO_2 enhanced platelet counts and preserved platelet morphology during storage

Platelet activation and apoptosis were assessed to evaluate the effects of a CO_2-enriched environment during storage. The data revealed that CO_2 exerted a protective effect against platelet activation, with 3% identified as the optimal concentration in the current preservation system (Supplementary Figure S1). Additionally, the protective effects of CO_2 against storage-related lesions were assessed by analyzing fundamental platelet properties. Platelet counts stored under CO_2 conditions for 3, 5, and 7 days were comparable with those in the control group (Figure 1A). However, the mean platelet volume was notably lower in the 3% CO_2 group on days 5 and 7 (Figure 1B). SEM analysis revealed well-preserved platelet morphology in the 3% CO_2 groups compared with the control group, which exhibited pseudopodia extension and increased spinous platelets (Figures 1C, D). These findings collectively suggest that platelets are better preserved in 3% CO_2.

3.2 | CO_2 helped maintain platelet function in hemostasis *in vitro*

Analysis of platelet coagulation function *in vitro* revealed a decline in adhesion ability in the control group with prolonged storage.

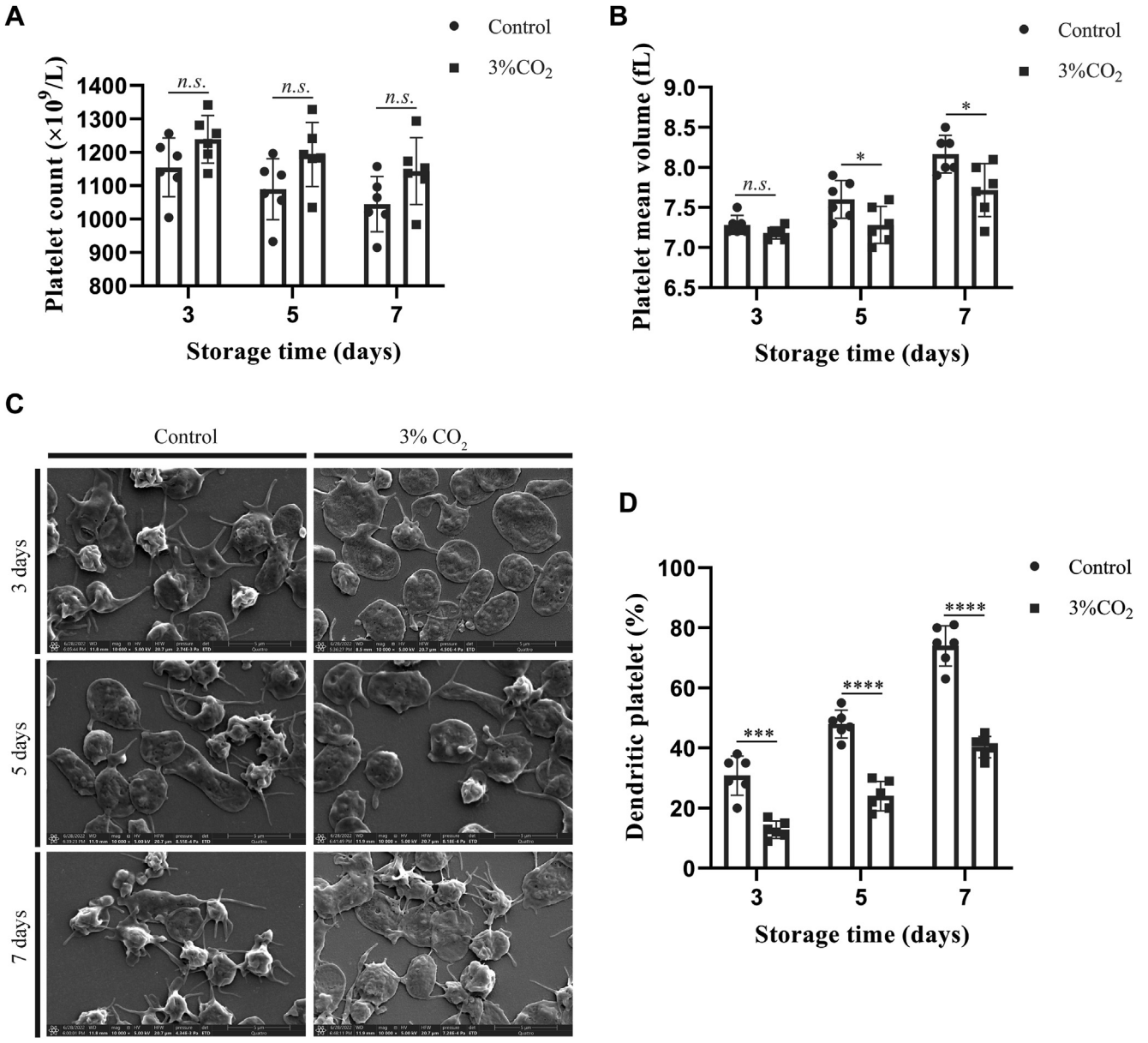


FIGURE 1 Carbon dioxide (CO_2) improved the counts and morphology of platelets. The evaluation of platelet parameters included (A) platelet count and (B) platelet mean volume. (C) Representative micrograph of platelets under different conditions, scale bar = 5 μm. Statistical analysis of dendritic platelets is presented in (D). The results are expressed as mean ± SEM (n = 6). *n.s.*, not significant, $^*P < .05$, $^{***}P < .001$, $^{****}P < .0001$.

In contrast, platelets stored with 3% CO_2 exhibited enhanced adhesion to collagen- and fibrinogen-coated surfaces (Figure 2A–C). Furthermore, arachidonic acid and adenosine diphosphate were utilized as activators to assess platelet aggregation. The maximum platelet aggregation rate induced by adenosine diphosphate and arachidonic acid was significantly higher in the 3% CO_2 group than the control group (Figure 2D–F). Additionally, platelet clot contraction, evaluated using 0.5 U/mL thrombin, revealed that clot retraction time in the CO_2 group was consistently limited to 10 minutes, regardless of storage duration (3, 5, or 7 days). In contrast, clot retraction times in the control group extended to 20 minutes on day 5 and exceeded 20 minutes on day 7 (Figure 2G–J). These findings suggest that CO_2 supplementation enhances the preservation of platelet clotting function *in vitro*.

3.3 | CO_2 improved platelet survival and coagulation function *in vivo*

A crucial aspect of platelet transfusion is the retention rate *in vivo*. Human platelets stored with CO_2 for 5 days exhibited significantly higher retention rates in NOD-SCID mice, with 40% retention at 0.5 hours and 30% at 2 hours posttransfusion compared with 23% and 18% in the control group, respectively (Figure 3A–C). These results

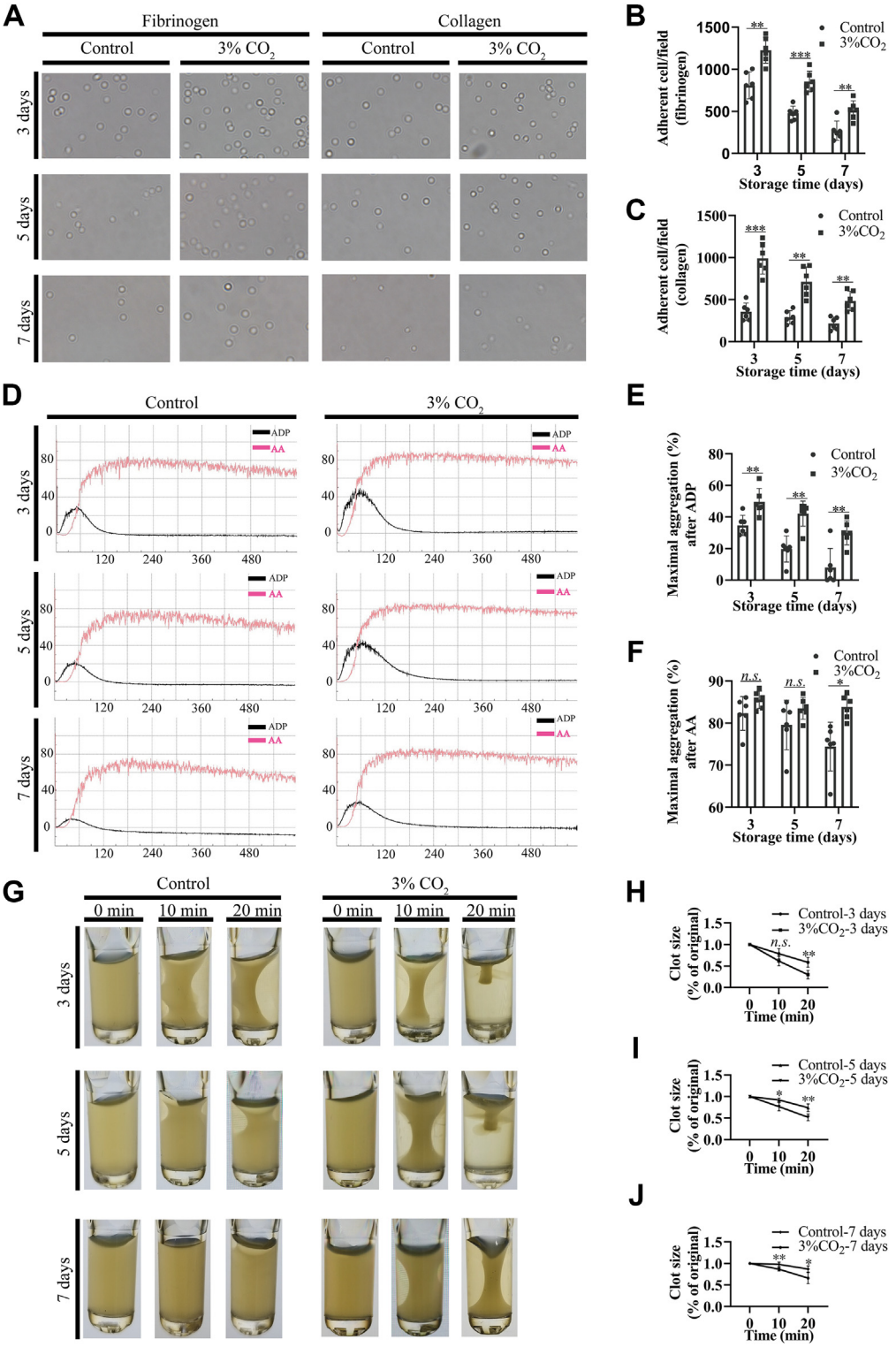


FIGURE 2 Carbon dioxide (CO_2) enhanced the adhesion, aggregation, and contraction functions of stored platelets. (A) Representative images of platelet adhesion. Statistical analysis of the adhesive platelets with (B) fibrinogen and (C) collagen. (D) Characteristic examples of platelet aggregation curves in different conditions. Statistical analysis of maximum platelet aggregation rate with (E) adenosine diphosphate (ADP) and (F) arachidonic acid (AA). (G) Representative images showing blood clots under different conditions. (H–J) The size of thrombi generated by samples under different conditions. The results are expressed as mean ± SEM ($n = 6$). *n.s.*, not significant, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

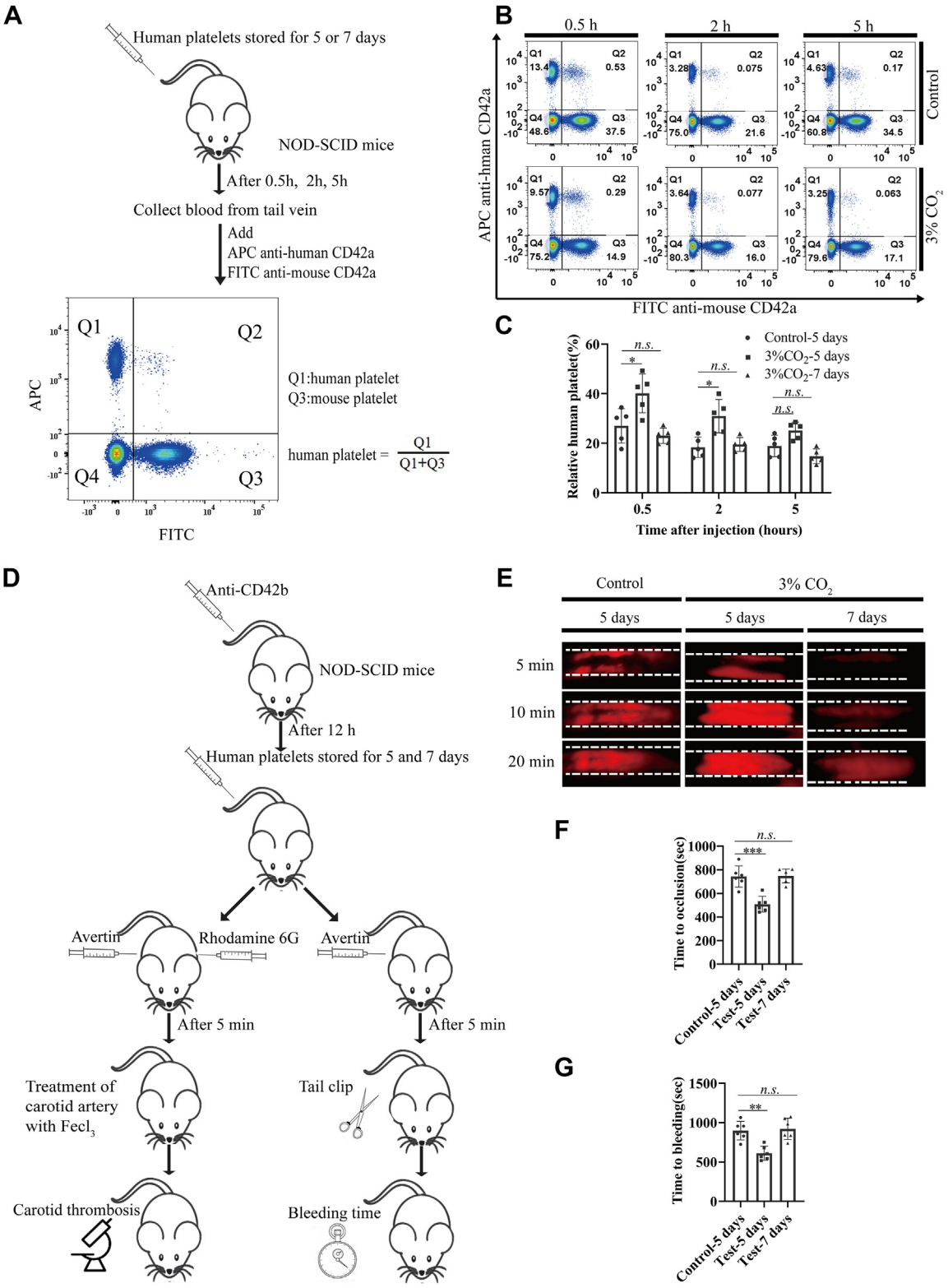


FIGURE 3 Carbon dioxide (CO_2) improved platelet survival and coagulation function *in vivo*. (A) Protocol overview of the human platelet transfusion experiment. (B, C) Human platelets (APC antihuman CD42a-positive [APC]) were transfused into NOD-SCID mice, and their survival rate was determined by flow cytometry. (D) Protocol overview of the human platelet transfusion experiment. (E) Representative images of thrombosis under different conditions. (F) Thrombosis duration in NOD-SCID mice. (G) Bleeding time in NOD-SCID mice. The results are expressed as mean ± SEM ($n = 6$). *n.s.*, not significant, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. FITC, FITC antimouse CD42a; Q, quadrant.

underscore the significant enhancement of posttransfusion platelet performance with CO_2 treatment. Coagulation function analysis revealed that mice transfused with platelets stored under CO_2 conditions for 5 days demonstrated earlier arterial thrombosis onset and improved thrombus strength compared with the control group (Figure 3D–F). Bleeding time assays corroborated these findings, showing significantly shorter bleeding times in mice transfused with platelets stored with CO_2 for 5 days compared with the control group (Figure 3G). These collective observations highlight the substantial preservation of platelet coagulation function *in vivo* with CO_2 supplementation.

3.4 | CO_2 inhibited platelet activation and apoptosis during storage

Flow cytometry and ELISA assessed platelet activation and apoptosis during storage. Over time, both groups exhibited elevated levels of CD62P and increased PF4 release, indicating platelet activation (Figure 4A, B). Concurrently, apoptosis increased, while CD42b levels decreased in stored platelets (Figure 4C, D). However, platelets stored with CO_2 demonstrated significantly lower CD62P and PF4 release levels than the control group, irrespective of storage duration (Figure 4A, B). Moreover, CO_2-treated platelets exhibited significantly reduced annexin V levels and a slower decline in CD42b levels compared with the control group (Figure 4C, D). These findings suggest that CO_2 effectively inhibits platelet activation and apoptosis during storage.

3.5 | CO_2 reduced glucose consumption and lactic acid accumulation in platelet storage

Given the importance of glucose metabolism and lactic acid accumulation in platelet storage, we investigated alterations in platelets' glucose and lactic acid levels. Glucose levels in the CO_2-treated group were significantly higher than in the control group on days 5 and 7 (Figure 5A), indicating reduced glucose consumption. Conversely, lactic acid levels were significantly lower in the CO_2 group than in the control group (Figure 5B), suggesting reduced lactic acid accumulation. Given the elevated CO_2 levels in the storage environment, we measured CO_2 levels. The results revealed a significant increase in CO_2 levels (Figure 5C). Despite a slight decrease, the pH levels remained within the normal range of 6.8 to 7.4, likely due to increased dissolved CO_2 levels and reduced lactic acid production (Figure 5D). Notably, dissolved O_2 concentrations remained stable in the presence of CO_2 (Figure 5E). Additionally, HCO_3^- levels were significantly higher in the CO_2 group than in the control group, which may help maintain the pH levels of the storage system (Figure 5F). These findings demonstrate that CO_2 reduced glucose consumption and lactic acid accumulation in platelets.

3.6 | CO_2 improved the platelet fatty acid metabolism

Building on preliminary experiments, we collected platelets on the third day of storage for metabolomics analysis. Principal component analysis revealed significant differences between the 3% CO_2 and control groups (Figure 6A). Volcano plots further emphasized significant differences in metabolite profiles between the 2 groups (Figure 6B), with 15 metabolites showing significant alterations in the 3% CO_2 group ($P < .05$; Figure 6C). Notably, fatty acid oxidation metabolites, such as 3-hydroxybutyric acid, 3-methyl-2-oxovaleric acid, alpha-ketoisovaleric acid, and methylmalonic acid, were elevated in the 3% CO_2 group (Figure 6D, F–H). Conversely, dicarboxylic acids, including suberic acid, azelaic acid, and pimelic acid, were significantly reduced (Figure 6E, I–J). Additionally, total free fatty acid levels in the supernatant were significantly reduced in the presence of CO_2 (Figure 6K), indicating enhanced fatty acid metabolism in platelets. Signal pathway analysis suggested that high CO_2 treatment induced metabolic shifts involving ketone body synthesis and degradation and valine, leucine, and isoleucine metabolism (Figure 6L). These data indicate that CO_2 enhanced fatty acid metabolism in platelets during storage.

3.7 | CO_2 inhibited p38 phosphorylation in platelets

Subsequently, ATP production in platelets was examined to evaluate energy supply during storage. ATP concentrations were significantly higher in the CO_2 group than the control group during storage, suggesting a metabolic shift or the utilization of more efficient energy substrates (Figure 7A). Biochemical analyses revealed a significantly lower adenosine monophosphate-to-ATP ratio in platelets from the CO_2 group than the control group (Figure 7B), indicating enhanced energy production efficiency. Phospho-p38 levels, a marker of platelet activation, were significantly lower in the CO_2 group than the control group on days 3, 5, and 7. Notably, these levels were comparable with or lower than those in the p38 mitogen-activated protein kinase (MAPK) inhibitor VX702-treated group, particularly on day 7 (Figure 7C, D). These findings underscore the potential of CO_2 to enhance platelet quality during storage.

4 | DISCUSSION

The present study demonstrated that elevated CO_2 levels during platelet storage effectively safeguarded platelet viability and functionality. The heightened CO_2 environment notably inhibited platelet activation and apoptosis during storage. Subsequent analyses revealed that 3% CO_2 effectively reduced glucose consumption and lactic acid accumulation in platelets while facilitating normal fatty acid oxidation. These findings collectively highlighted CO_2 as a promising strategy for platelet storage and quality preservation over extended periods.

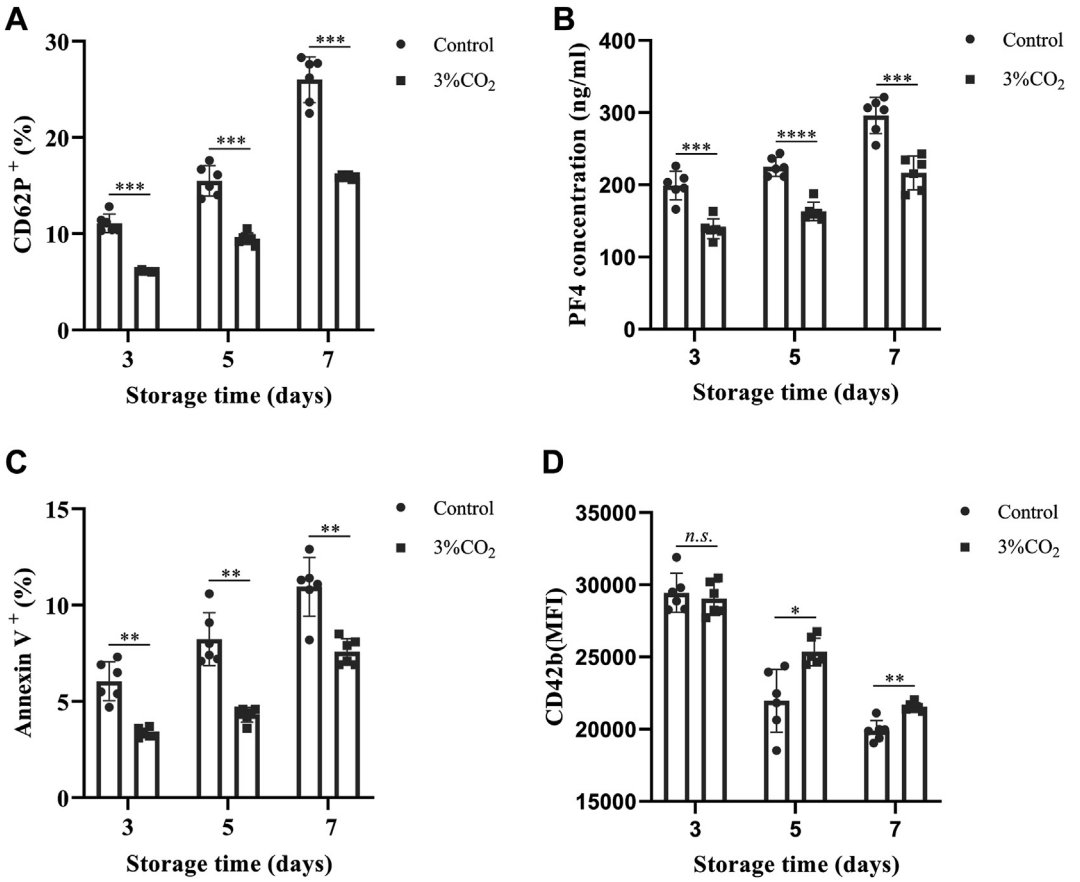


FIGURE 4 Carbon dioxide (CO_2) inhibited platelet activation and apoptosis during storage. (A) The percentage of CD62P-positive platelets was determined by flow cytometry. (B) Platelet factor 4 (PF4) concentration was determined by enzyme-linked immunosorbent assay. (C) The percentage of annexin V-positive platelets was determined by flow cytometry. (D) The mean fluorescence intensity (MFI) of CD42b was determined by flow cytometry ($n = 6$). *n.s.*, not significant, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

The accumulation of lactic acid during platelet storage, which decreases pH levels, is a critical factor limiting storage [12,21]. By elevating CO_2 concentration in the platelet storage system, we effectively maintained low levels of lactic acid and high HCO_3^- levels, mitigating the risk of rapid acidification. Elevated CO_2 concentration benefits the physiological state of platelets and enhances storage system stability. Evidence suggests that CO_2 protects various organs primarily by modulating cellular metabolism [22,23]. For instance, in hypercapnia induced by intestinal endotoxin, CO_2 prevents ATP depletion and lactic acid accumulation while promoting fatty acid metabolism in macrophages and bronchial epithelial cells, contributing to organ protection [18–20]. Fatty acid β-oxidation is blocked during platelet preservation *in vitro*, resulting in lactic acid accumulation, which can damage mitochondrial structure and induce platelet apoptosis [24]. Our data demonstrated that elevated CO_2 levels effectively reduced glucose consumption and enhanced fatty acid metabolism in platelets, improving their storage quality. Additionally, many studies show that high CO_2 levels enhance metabolite levels, modulate fatty acid metabolism, and promote oxidative phosphorylation, supporting the benefits of elevated CO_2 on cellular metabolism [25,26].

Our findings revealed elevated metabolites linked to fatty acid oxidation, such as hydroxybutyric acid, 3-methyl-2-oxovaleric acid, alpha-ketoisovaleric acid, and methylmalonic acid, alongside reduced dicarboxylic acid products, including suberic acid, azelaic acid, and pimelic acid. Dicarboxylic acids, derived from medium- and long-chain monocarboxylic fatty acids through Ω-oxidation, likely increase in response to lipid peroxidation [27–29]. This suggests that elevated CO_2 may promote fatty acid metabolism, but further investigation is needed to determine whether mitochondrial function in platelets remains intact. While elevated CO_2 reduces glucose consumption and lactic acid accumulation, the metabolic shift from glycolysis to oxidative phosphorylation remains unclear and requires further study.

PSLs are associated with the activation of p38-MAPK [30]. Prolonged storage induces mitochondrial injury, which triggers p38 phosphorylation in platelets. Platelet agonists activating apoptosis signal-regulating kinase 1 (ASK1) can trigger p38-MAPK–mediated activation of integrin $\alpha_{IIb}\beta_3$ and platelet aggregation, resulting in thromboxane A2 production, which is crucial for thrombosis [31]. Inhibiting the p38-MAPK pathway significantly prolongs platelet recovery and hemostasis after transfusion in mice. Although the limited specificity of p38-MAPK inhibitors complicates conclusions about

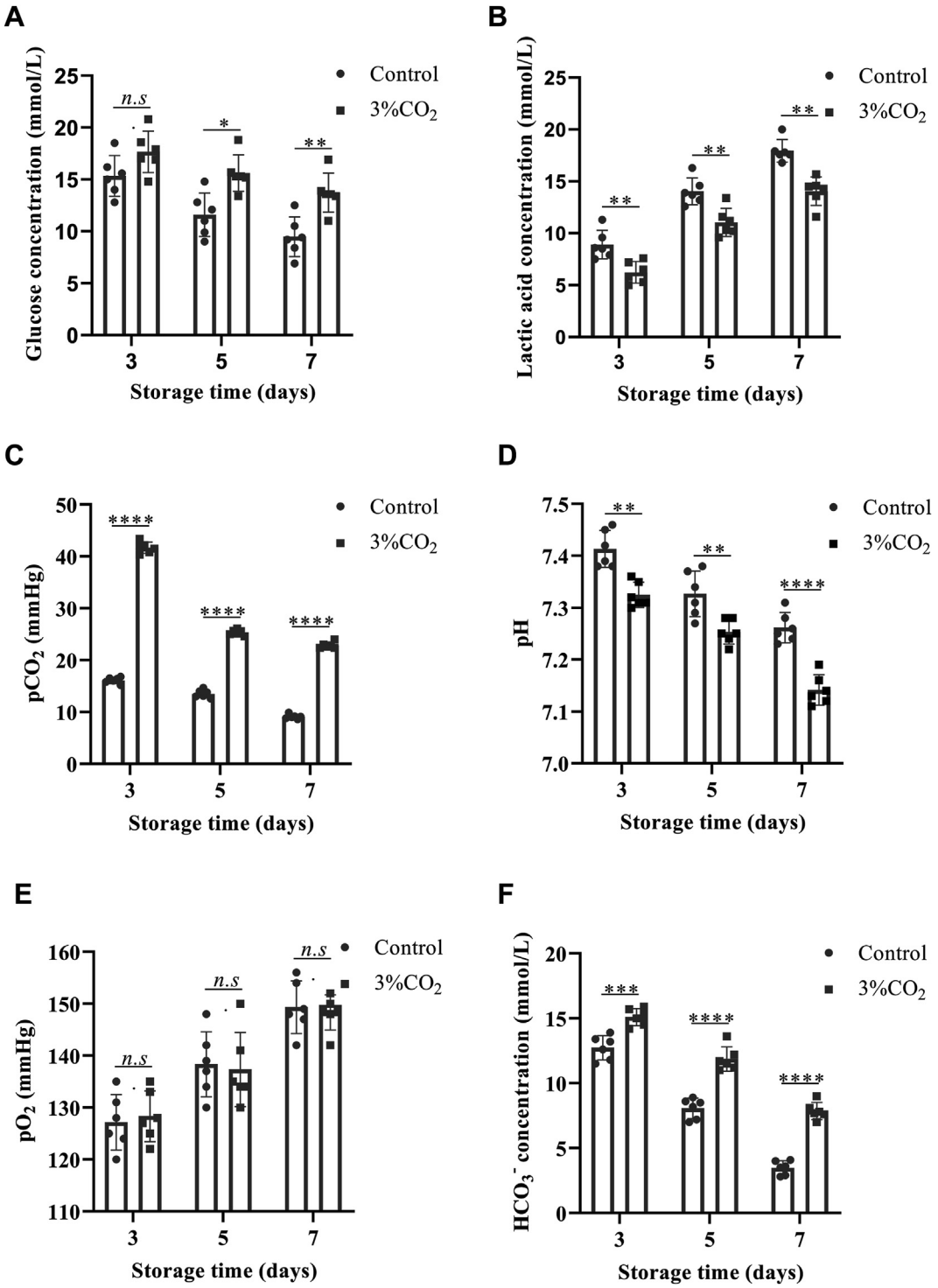


FIGURE 5 Carbon dioxide (CO_2) reduced glucose consumption and lactic acid accumulation in platelet storage. (A) The glucose concentration, (B) lactic acid concentration, (C) CO_2 partial pressure (pCO_2), (D) pH, (E) oxygen partial pressure (pO_2), and (F) bicarbonate (HCO_3^-) concentration were detected by blood gas analysis. The results are expressed as mean ± SEM (n = 6). *n.s.*, not significant, $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

their role in platelet function regulation, VX702, a classic p38-MAPK inhibitor, effectively preserves platelet storage parameters *in vitro*, particularly mitochondrial function [32].

Our findings demonstrated that 3% CO_2 significantly reduces p38 activation, surpassing the efficacy of VX702 under normal conditions. This highlights the protective effects of CO_2 on stored platelets and its

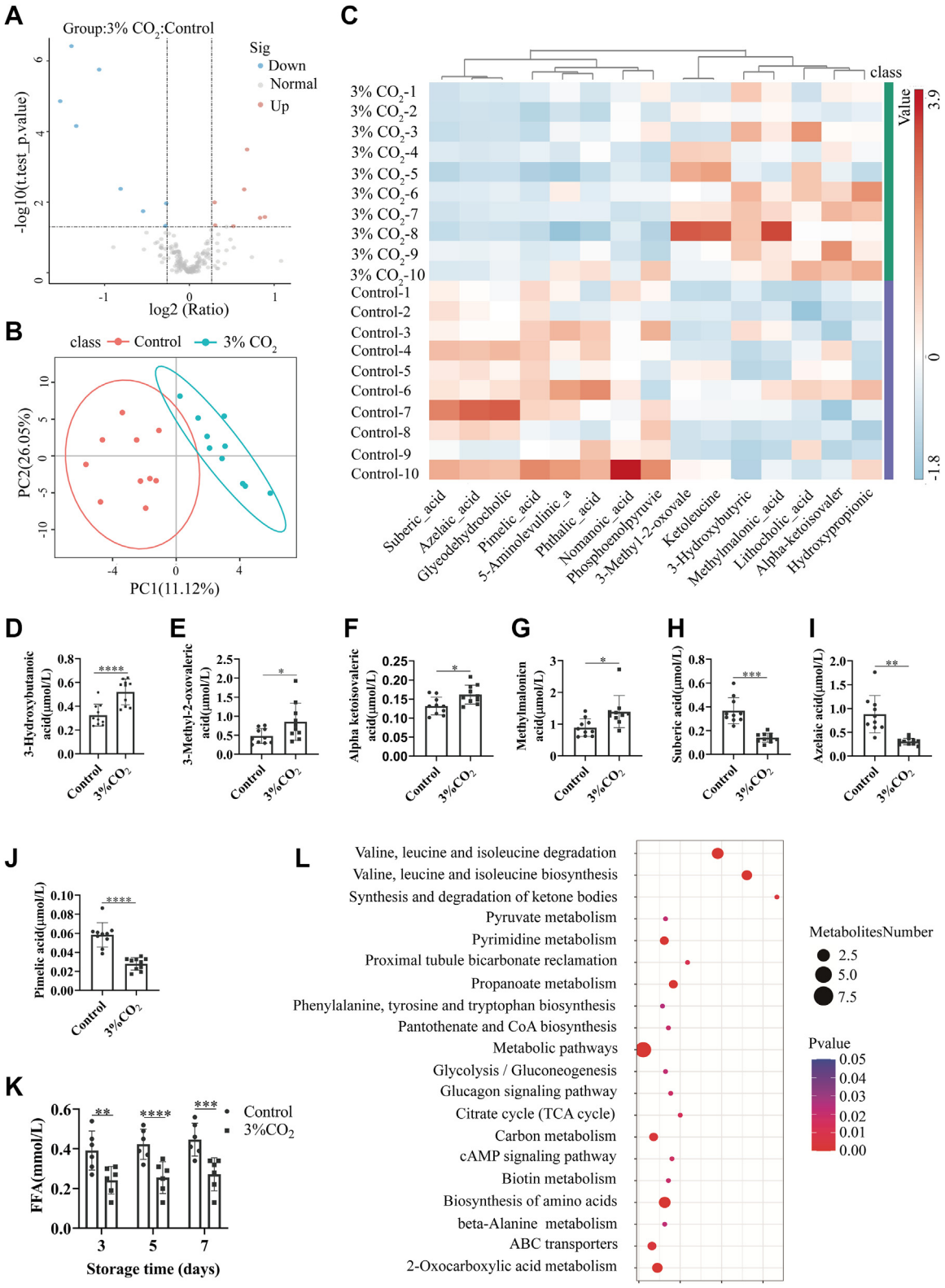


FIGURE 6 Carbon dioxide (CO_2) improves the metabolism of free fatty acids (FFAs) during platelet storage. (A) Volcano plot and (B) partial least squares discriminant analysis score of platelet metabolism analysis in the control and 3% CO_2 group (n = 10). (C) Differentially metabolism compounds between the control and 3% CO_2 group (n = 10). Statistical analysis of the concentration of (D) 3-hydroxybutyric acid, (E) 3-methyl-2-oxovaleric acid, (F) alpha-ketoisovaleric acid, (G) methylmalonic acid, (H) suberic acid, (I) azelaic acid, and (J) pimelic acid (n = 10). (K) Statistical analysis of the concentration of FFAs (n = 6). (L) Kyoto Encyclopedia of Genes and Genomes analysis of differentially expressed metabolites between the control and 3% CO_2 group (n = 10). The results are expressed as mean ± SEM. *P < .05, **P < .01, ***P < .001, ****P < .0001. cAMP, cyclic adenosine monophosphate; PC, principal component.

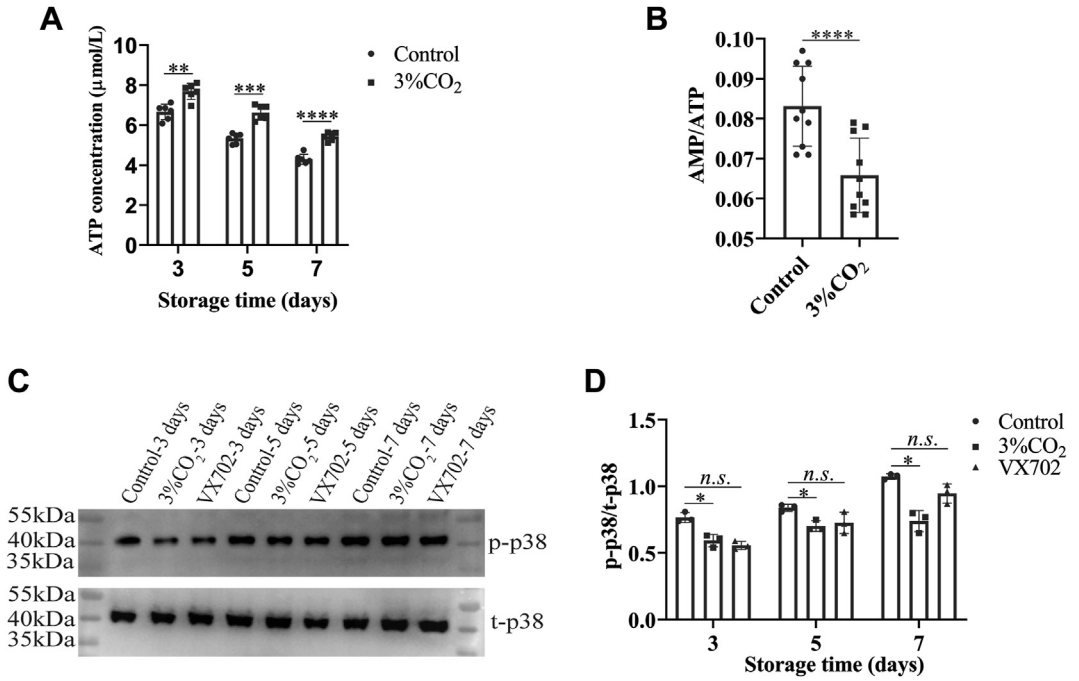


FIGURE 7 Carbon dioxide (CO_2) inhibited the activation of the p38 mitogen-activated protein kinase signaling pathway during platelet storage. (A) The concentration of adenosine triphosphate (ATP) in the control and 3% CO_2 group. (B) The ratio of adenosine monophosphate and ATP concentrations. (C) Western blot analysis of phospho-p38 (p-p38) and total p38 (t-p38) of platelets. (D) Quantification of p-p38 and t-p38 of platelets. The results are expressed as mean ± SEM (n = 6). *n.s.*, not significant, *P < .05, **P < .01, ***P < .001, ****P < .0001. VX702, p38 mitogen-activated protein kinase inhibitor.

potential utility for platelet storage. Therefore, we propose that CO_2 aids platelets in maintaining energy supply by stimulating fatty acid metabolism and reducing glucose consumption. Suppressing p38 pathway activation is one mechanism by which platelets maintain their active state in CO_2-enriched environments.

However, our findings are based solely on *in vitro* experiments, which may not fully replicate the complexities of clinical or *in vivo* settings. In addition, the study involved platelets from only 28 healthy donors, which may not account for variability in platelet quality or behavior across diverse populations or pathological conditions.

In summary, our study highlights the significant benefits of increasing CO_2 concentration in the storage environment to preserve platelets. However, the findings of this study are limited to *in vitro* conditions and may not fully translate to clinical settings. Further research should investigate the clinical applicability of CO_2-enriched storage environments.

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AUTHOR CONTRIBUTIONS

S.G., J.X., X. Hou, X. Hu, and W.Y. designed the experiments. S.G. performed most of the experiments and analyzed the data. E.L., N.A., Y.C., Z.L., and W.W. assisted in the experiments. J.X. and X. Hou wrote and revised the manuscript. S.G. and W.Y. provided funding support. All authors contributed substantially to this research. All authors read and approved the final manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

ORCID

Shunli Gu https://orcid.org/0000-0002-1201-1845

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SUPPLEMENTARY MATERIAL

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