Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Therapeutic Effect of Myostatin/Activin Blockage and Dietary Restriction in Modulation of Platelet Activation Markers of Progeroid Mouse Model

Khalid Alyodawi

Department of Anatomy and Medical Biology, College of Medicine, Wasit University, IRAQ
Corresponding Author: Khalid Alyodawi

DOI: https://doi.org/10.31185/wjps.833
Received 21 April 2025; Accepted 07 July 2025; Available online 30 June 2026

ABSTRACT: Aging-related decline in hematopoietic and platelet function is a hallmark of DNA repair deficiency syndromes, yet the impact of metabolic and pharmacological interventions on platelet biology in this context remains unclear. This study addresses this gap by investigating whether dietary restriction (DR) and myostatin/activin blockade through soluble Activin Receptor Type IIB (sActRIIB) can modulate platelet activation markers—GPIbα and P-Selectin—in a progeroid mouse model (*Ercc1*$^{\Delta/-}$). While DNA repair-deficient mice exhibited significantly reduced GPIbα expression compared to wild-type controls, sActRIIB treatment showed a modest, though non-significant, improvement. In contrast, DR alone or combined with sActRIIB had negligible effects. P-Selectin levels remained unchanged between mutant and control mice; however, sActRIIB treatment significantly suppressed its expression, indicating a potential anti-inflammatory effect. These findings suggest that myostatin/activin pathway inhibition may offer a partial therapeutic benefit in modulating platelet activation in progeroid models, whereas dietary restriction appears insufficient. It is important to conduct further studies to investigate specific, combinatorial application in the restoration of platelet homeostasis in DNA repair-deficient aging diseases.

Keywords: *Ercc1*$^{\Delta/-}$ mice, Dietary restriction, Myostatin/activin blockade, GPIbα, P-Selectin

1. INTRODUCTION

Progeroid mice, deficient in the major DNA repair proteins, including *Ercc1*$^{\Delta/-}$, are powerful models of rapid ageing. These mice have the hallmarks of premature ageing, such as sarcopenia, genomic instability, impaired stem cell functioning, and shortened lifespan [1]. The *Ercc1*$^{\Delta/-}$ model is of special value in ageing studies, since it recapitulates numerous phenotypes previously seen in ageing humans, thus furnishing the opportunity to examine molecular processes involved in age-related decay. Significantly, these mice offer an advantageous model through which the impacts of therapeutic strategies (such as dietary restriction or genetic manipulation) can be studied on different physiological systems challenged by ageing. Both recapitulated systemic and tissue-specific age-related phenotypes of *Ercc1*$^{\Delta/-}$, and this model allows dissection of the mechanistic interplay between genomic instability and cellular senescence, inflammation, metabolism and tissue regeneration. In this way, their employment in the case is an important fact to determine the impact of DNA repair deficiency in platelet biology and general age-related diseases [1].

Dietary restriction (DR) may be considered one of the most researched non-pharmacological interventions that has been shown to increase both longevity and health span across a number of different species [2]. DR has been proven to increase resistance to oxidative stress, decrease the prevalence of age-related diseases and improve metabolic efficiency and immune performance. It also activates critical DNA repair signalling pathways such as mitochondrial processes, essential in sustaining genomic integrity in the ageing organism [3]. In addition to its systemic effects, DR alters inflammation and reduces oxidative damage, which is at the core of ageing and age-related pathology. However, the specific influence of DR on platelet-related biomarkers—particularly GPIbα and P-Selectin—remains unclear. GPIbα, for example, plays a role in platelet activation and monocyte recruitment during systemic inflammation, linking platelet

*Corresponding author: khthamer@uowasit.edu.iq

function to age-associated inflammatory pathways [4]. This gap in knowledge warrants further investigation, especially in models of genomic instability where platelet dysfunction contributes to age-related pathology.

Recent research suggests that dietary restriction (DR) may work synergistically with interventions that promote muscle health, such as myostatin/activin inhibition, to enhance systemic outcomes in ageing models [5]. These combined approaches have shown promise in improving muscle mass, reducing inflammation, and extending health span in DNA repair-deficient mice. While DR alone has been effective in delaying tumorigenesis and attenuating oxidative damage, the biological effects of its combination with pharmacological agents on platelet activation markers remain largely unstudied [6]. Platelets are increasingly recognised as key players in ageing due to their role in inflammation, vascular dysfunction, and thrombosis. Despite this, the effects of DR and myostatin/activin blockade on platelet function in progeroid contexts have not been thoroughly explored. Platelets play a crucial role in vascular homeostasis, but in ageing, they contribute to chronic inflammation, thrombosis, and atherosclerosis. Age-related platelet hyperactivity is associated with increased cardiovascular risk and impaired hemostatic balance, especially in diseases such as stroke, myocardial infarction, and metabolic syndrome [7].

GPIbα and P-Selectin are critical platelet surface markers involved in adhesion and activation, and they are known to be dysregulated in both ageing and DNA repair deficiency states [8, 9]. GPIbα is a glycoprotein encoded by the GP1BA gene and is primarily expressed on the surface of platelets. It serves as a receptor for von Willebrand factor and is critical for platelet adhesion to vascular endothelium. Conversely, P-Selectin is a cell adhesion molecule stored in platelet alpha granules and endothelial Weibel-Palade bodies, rapidly translocated to the surface upon activation. Both proteins are key platelet activation markers commonly used to assess thrombo-inflammatory status in ageing and disease [10]. Emerging evidence highlights that metabolic interventions may differentially regulate these markers; for instance, DR may affect hematopoietic stem cell ageing and platelet production through genetically regulated mechanisms [11]. However, no previous study has directly examined how DR and myostatin/activin inhibition individually or in combination modulate GPIbα and P-Selectin expression in *Ercc1*$^{\Delta/-}$ mice. The lack of studies presents a clear gap in understanding the interface between metabolism, platelet biology, and genomic instability. By addressing this gap, our study seeks to clarify whether targeting systemic muscle signalling pathways or nutrient sensing mechanisms can impact platelet homeostasis in models of accelerated ageing.

Given the pivotal role of platelet activation in age-related inflammation and vascular pathology, understanding how metabolic and pharmacological interventions influence platelet biomarkers in the context of DNA repair deficiency is essential. Despite evidence supporting the benefits of dietary restriction and muscle-promoting therapies in systemic ageing, their effects on platelet function remain poorly understood, particularly in progeroid models such as *Ercc1*$^{\Delta/-}$ mice. sActRIIB-Fc is a soluble chimeric fusion protein and a decoy receptor that binds activin and myostatin ligands, interfering with ligand-receptor interactions. Such a blockade has been reported to stimulate muscle growth and limit inflammation, and can act tilting on other signalling systems that have systemic effects on platelet function [12]. This research has filled this critical knowledge gap to investigate how exposure to dietary restriction (DR) and inhibition of Myostatin or Activin using soluble Activin Receptor Type IIB (sActRIIB) affect the expression of two prominent markers on platelets, GPIb alpha and P-Selectin. We surmise that equivalent immunohistochemical approaches in a genetically legitimate system of accelerated ageing will reveal that these interventions may affect platelet activation and lead to healthier ageing phenotypes. In the present study, the glycoprotein binding protein (GPIb alpha) and P-Selectin expression were identified by antigen-antibody interaction via the immunohistochemical method, in which spatial identification of surface proteins was expressed by particular primary antibodies and labelled secondary antibodies. In this way, the study aims at expanding our cognisance of the tangential interaction between two diverse metabolisms and signalling pathways with the essence of hematopoietic impairment under the circumstances of DNA repair deficiency, thus providing new knowledge in therapeutic strategies directed towards

2. MATERIALS AND METHODS

2.1 STUDY ANIMALS AND CARE

A total of 40 *Ercc1*$^{\Delta/-}$ mice and their wild-type (WT) littermates (aged 8 weeks at study initiation) were used in this study. All animals were male, weighing between 20 and 25 grams, and were bred and genetically validated by the Erasmus Medical Centre (Rotterdam, The Netherlands), where heterozygous *Ercc1*$^{+/-}$ mice were crossed to generate *Ercc1*$^{\Delta/-}$ offspring as previously established [13]. The experiments were carried out in compliance with the Dutch Ethical Committee's instructions, the Principles of Laboratory Animal Care, and all applicable European laws (permits #139-12-13, 139-12-18, and 18-6886-05).

The animals were housed in individually ventilated cages under specific-pathogen-free (SPF) conditions, with a 12-hour light/dark cycle, temperature maintained at 20–22°C, and ad libitum access to sterile water. Mice were randomly distributed into five experimental groups (n = 8 per group). Animal health was monitored daily, and body weight was recorded weekly throughout the study.

2.2 DIETARY RESTRICTION (DR) AND SACTRIIB INTERVENTIONS

2.2.1 DIETARY RESTRICTION (DR):

Mice assigned to the DR groups received 70% of the control mice's average daily food intake (i.e., 1.6 g/day vs. 2.3 g/day). DR began at 8 weeks and continued throughout the 8-week experimental period. Standard rodent chow was provided (Envigo, UK).

2.2.2 MYOSTATIN/ACTIVIN BLOCKADE (SACTRIIB-FC):

Mice in the sActRIIB groups were treated with a recombinant fusion protein (sActRIIB-Fc) obtained from Acceleron Pharma Inc., Cambridge, MA, USA. The protein was administered via intraperitoneal injection at a dose of 10 mg/kg body weight, twice weekly for 8 weeks.

2.3 STUDY DESIGN

The experiment was done on an 8-week duration and using five experimental groups namely wild-type (WT) control which was untreated, *Ercc1*$^{\Delta/-}$ untreated control, *Ercc1*$^{\Delta/-}$ treated with dietary restriction (DR) *Ercc1*$^{\Delta/-}$ treated with soluble Activin Receptor Type IIB (sActRIIB) and the *Ercc1*$^{\Delta/-}$ treated with a combination of dietary restriction. All the mice were euthanised at the end of the intervention period using CO_2 and cervical dislocation as per the animal care procedures of our institution immediately after gastrocnemius muscle tissues were harvested, frozen and preserved in liquid nitrogen-cooled isopentane and kept away in -80 C to carry out histological studies in the laboratory later.

2.4 TISSUE PROCESSING AND MUSCLE CRYOSECTIONING

Euthanasia of all mice was performed following the specified ethical regulations, i.e., through the use of CO_2 asphyxiation and subsequent cervical dislocation. Immediately, the gastrocnemius muscle was dissected, snap-frozen in liquid nitrogen-cooled isopentane and embedded in OCT LT Embedding Compound (TAAB O023, TAAB Laboratories, UK). The tissue blocks were immediately embedded and stored in a refrigerator at -80°C.

Cryosections were prepared using a Leica CM1950 cryostat (Leica Microsystems, Germany) set at –20°C. Muscle tissue was sectioned at a thickness of 10 µm and mounted on SuperFrost Plus slides (Thermo Fisher Scientific, USA). Sections were air-dried for 30 minutes at room temperature before proceeding to immunohistochemical staining.

2.5 IMMUNOHISTOCHEMISTRY PROTOCOL

Sections were rehydrated in phosphate-buffered saline (PBS, pH 7.4) for 10 minutes. Non-specific binding sites were blocked using a blocking buffer of PBS supplemented with 5% fetal calf serum (FCS, v/v) and 0.05% Triton X-100 (Sigma-Aldrich, Germany), incubated for 1 hour at room temperature.

Primary antibodies to detect GPIbα and P-Selectin were applied in blocking buffer and incubated overnight at 4°C in a humidified chamber. After washing in PBS (3 × 5 minutes), secondary antibodies were applied for 1 hour at room temperature, followed by further PBS washes. Nuclei were counterstained using DAPI (1 µg/mL; Sigma-Aldrich, Germany). Primary and secondary antibodies are described in Table 1. Immunofluorescent-stained sections were examined using a Zeiss Axiolmager A1 fluorescence microscope, and pictures were taken with an Axiocam digital camera and Zeiss Axiovision computer software (version 4.8). Image analysis and quantification of fluorescence intensity were performed using ImageJ software (NIH, USA).

Table 1. - Primary and secondary antibodies used for immunohistochemistry.

Antibody Target	Species	Dilution	Supplier	Type
GPIbα (CD42b)	Rat	1:200	BioLegend	Primary
P-Selectin (CD62P)	Rabbit	1:100	Abcam	Primary
Anti-Rat IgG (Alexa Fluor 488)	Goat	1:500	Thermo Fisher Scientific	Secondary
Anti-Rabbit IgG (Alexa Fluor 594)	Donkey	1:500	Thermo Fisher Scientific	Secondary

2.6 STATISTICAL ANALYSIS

GraphPad Prism (San Diego, CA) version 10.1.0 was utilised for statistical studies. A one-way ANOVA with Tukey's multiple comparisons was used to compare genotypes and treatments across various groups. Individual data of mean (M ± SEM) are displayed in graphs. Statistical differences were considered significant when the p-value was less than 0.05 ($p < 0.05$).

3. RESULTS

GPIbα expression was recorded at 15.39 ± 0.46 in *Ercc1*$^{\Delta/-}$ mice and 21.85 ± 0.74 in wild-type (WT) controls, with a statistically significant difference between the groups ($p < 0.0001$). Following intervention, a modest elevation in GPIbα expression was observed in *Ercc1*$^{\Delta/-}$ mice treated with sActRIIB compared to their untreated counterparts. Although this increase suggested a trend toward restoration, it did not reach statistical significance. In contrast, dietary restriction (DR), whether administered alone or in combination with sActRIIB, produced only minimal changes in GPIbα levels, with no significant differences observed relative to the untreated *Ercc1*$^{\Delta/-}$ group. Notably, while both DR-treated groups exhibited slightly higher mean values than the untreated group, these variations remained statistically nonsignificant. These findings indicate that sActRIIB may have a limited effect on GPIbα restoration in this model, whereas DR alone or combined does not appear to influence its expression. Detailed group means and statistical comparisons are presented in Table 2 and visualised in Figures 1 and 2.

Table 2. The level of platelet GPIbα expression in various mouse groups

Group	Mean ± SEM
WT	21.85 ± 0.74
Ercc1$^{\Delta/-}$	15.39 ± 0.46
Ercc1$^{\Delta/-}$ sActRIIB	17.41 ± 0.88
Ercc1$^{\Delta/-}$ DR	15.92 ± 0.44
Ercc1$^{\Delta/-}$ sActRIIB/DR	16.24 ± 0.97

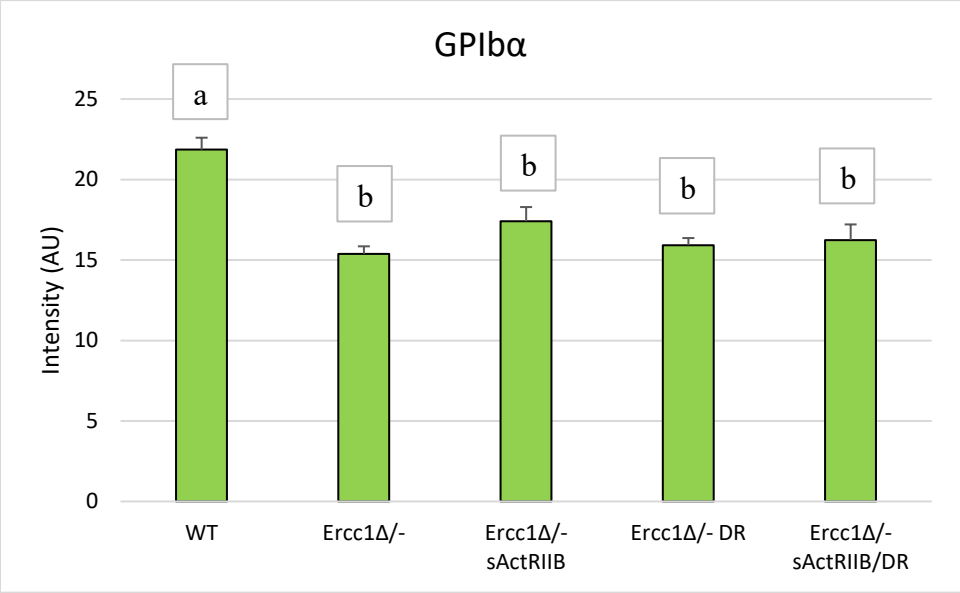

FIGURE 1. Quantitative comparison of GPIbα expression in wild-type and *Ercc1*$^{\Delta/-}$ mice under different treatments. Significant differences were observed between groups ($p < 0.05$). Groups not sharing the same letter are significantly different.

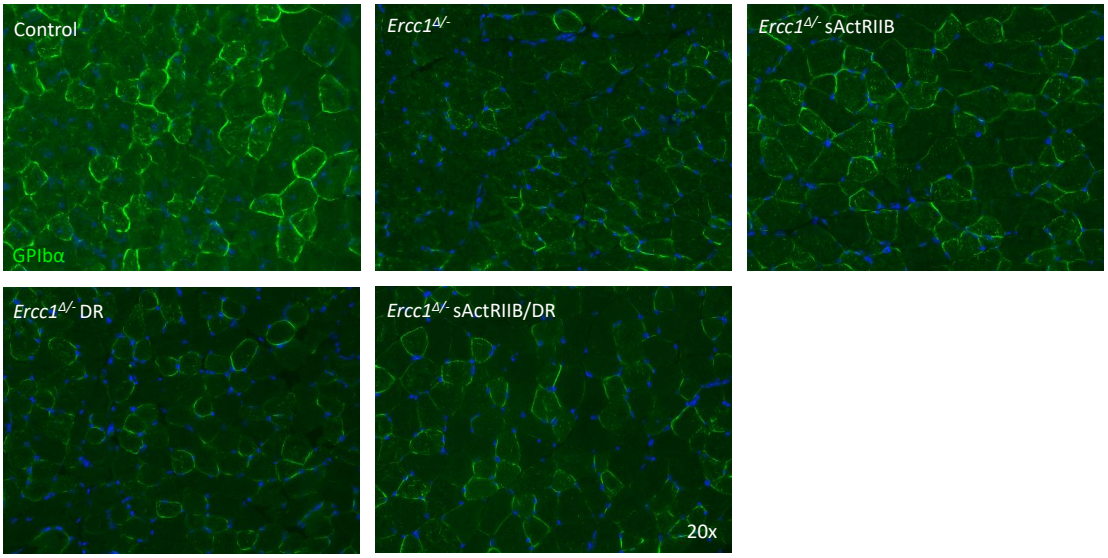

FIGURE 2. Representative immunofluorescence images of GPIbα expression in wild-type and *Ercc1*$^{\Delta/-}$ mice muscle tissue. Differences in GPIbα signal intensity reflect variable expression across treatment conditions.

P-Selectin expression was recorded at 6.11 ± 0.12 in wild-type (WT) mice and 6.52 ± 0.26 in *Ercc1*$^{\Delta/-}$ mice, with no statistically significant difference between the two groups ($p = 0.4841$). Treatment with sActRIIB significantly reduced P-Selectin expression in *Ercc1*$^{\Delta/-}$ mice, indicating a potential suppressive effect on platelet activation ($p = 0.0052$). In contrast, dietary restriction (DR), whether administered alone or in combination with sActRIIB, did not significantly alter P-Selectin levels compared to the untreated *Ercc1*$^{\Delta/-}$ group. Although the mean expression in the combined DR and sActRIIB group was slightly lower than DR alone, neither intervention alone nor in combination produced statistically significant changes. These findings suggest that while DR has minimal influence on P-Selectin expression in this progeroid model, sActRIIB treatment alone may exert a meaningful anti-inflammatory effect. Full quantitative results are provided in Table 3 and illustrated in Figures 3 and 4.

Table 3. - Mean ± SEM values of platelet P-Selectin expression in wild-type and *Ercc1*$^{\Delta/-}$ mice under different treatment conditions.

Group	Mean ± SEM
WT	6.11 ± 0.12
Ercc1$^{\Delta/-}$	6.52 ± 0.26
Ercc1$^{\Delta/-}$ sActRIIB	5.57 ± 0.10
Ercc1$^{\Delta/-}$ DR	6.30 ± 0.21
Ercc1$^{\Delta/-}$ sActRIIB/DR	5.97 ± 0.15

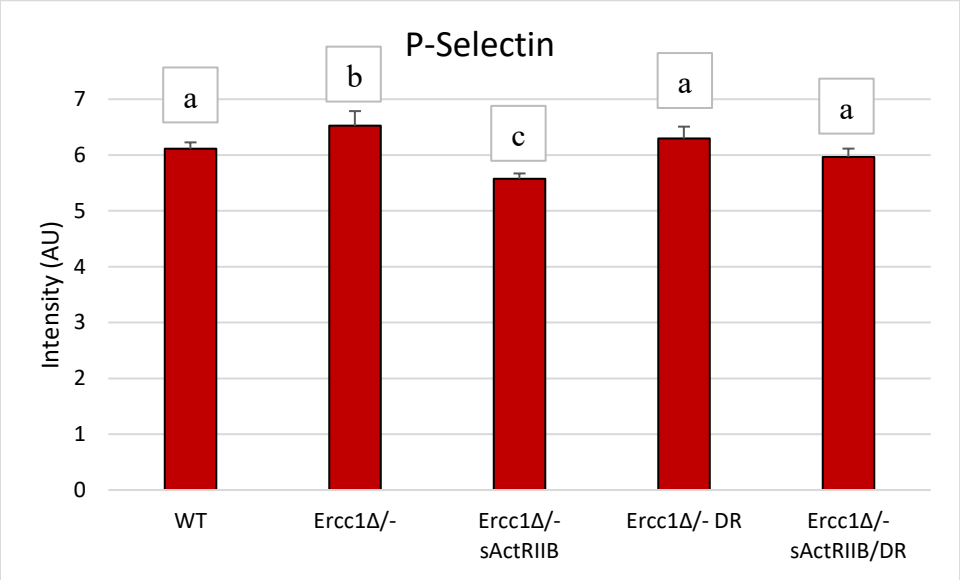

FIGURE 3. Quantitative analysis of P-Selectin expression in wild-type and *Ercc1*$^{\Delta/-}$ mice under different treatments. Statistical differences between groups are denoted by different letters ($p < 0.05$).

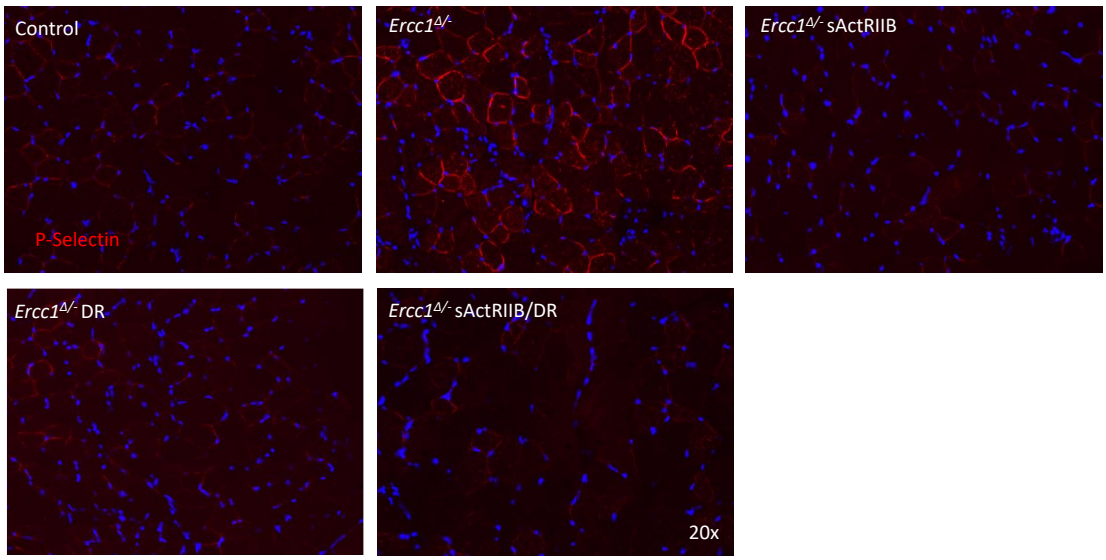

FIGURE 4. Representative immunofluorescence images of P-Selectin expression in wild-type and *Ercc1*$^{\Delta/-}$ mice muscle tissue. Images show variation in P-Selectin staining patterns among treatment groups.

4. DISCUSSION

A notable decrease in GPIbα expression was observed in *Ercc1*$^{\Delta/-}$ mice relative to wild-type controls, consistent with previous findings that link DNA repair deficiencies to impaired hematopoietic stem cell function and altered platelet biology [14]. This impairment is likely a consequence of genomic instability in bone marrow progenitors, adversely affecting megakaryopoiesis and platelet maturation [15]. Although not statistically significant, the observed trend of increased GPIbα expression following sActRIIB treatment implies that myostatin/activin blockade may indirectly support the hematopoietic niche. This result aligns with evidence that myostatin inhibition promotes the release of muscle-derived systemic factors capable of modulating stem cell activity [16]. These findings address an existing knowledge gap by illustrating that sActRIIB may influence platelet activation pathways, whereas dietary restriction appears insufficient to

elicit similar effects in this model. Collectively, the results emphasise the need for further investigation into the tissue-specific responsiveness of platelet biomarkers to different metabolic interventions.

Although P-Selectin expression was comparable between *Ercc1*$^{\Delta/-}$ and wild-type mice at baseline, a significant reduction was observed following sActRIIB treatment, indicating a potential regulatory effect of activin pathway inhibition on platelet activation. This observation is consistent with previous reports suggesting that activin signalling contributes to pro-inflammatory and pro-thrombotic processes, and that its blockade can attenuate these effects [17, 18]. Interestingly, dietary restriction (DR), whether applied alone or in combination with sActRIIB, did not elicit a comparable reduction in P-Selectin levels, contrasting with DR's known anti-inflammatory actions in other biological systems [19]. The difference can be explained by an overarching effect of chronic DNA repair deficiency in the *Ercc1*$^{\Delta/-}$ mice, supposedly disrupting platelet homeostasis and turnover to a degree surpassing the modulatory impact of metabolic interventions [20]. Since the *Ercc1*$^{\Delta/-}$ mice model most of the hallmarks of human premature ageing and DNA repair syndrome, the present observations justify the view that myostatin/activin pathway inhibition can be used as a potential therapeutic approach to address thrombotic risk and chronic inflammation in similar human conditions [21]. Such a strategy might have particular application to patients harbouring genetic damage to DNA repair systems, or patients afflicted with progeroid disorders whose diseases might be notably less amenable to standard metabolic disease treatment.

We found that dietary restriction (DR), given alone or combined with sActRIIB, did not significantly alter the GPIb expression and levels of P-Selectin in *Ercc1*$^{\Delta/-}$ mice. No significant trends were found in the DR+sActRIIB group, though small ones were seen. These findings indicate that DR, under the current timing and restriction level (30%), is insufficient to rescue platelet biomarker dysfunction in the context of DNA repair deficiency. This result may be due to the short lifespan and rapid turnover of platelets, which limits the systemic impact of metabolic interventions, or it could reflect a threshold effect requiring earlier or more prolonged treatment. Prior studies have shown that DR can extend lifespan and improve hematopoietic function in Ercc1-deficient models when initiated early [6, 19]. Therefore, future studies should explore varying levels of DR (e.g., 20% vs. 30%), earlier initiation, or extended intervention periods. In addition, further investigation into underlying mechanisms—such as thrombopoietin (TPO) signalling, platelet turnover, and hematopoietic niche integrity—may clarify how genomic instability alters platelet homeostasis and responsiveness to systemic interventions [22, 23].

5. STUDY STRENGTHS AND LIMITATIONS

This study benefits from several strengths. It employs a well-characterised and genetically validated model of accelerated ageing (*Ercc1*$^{\Delta/-}$ mice), replicating key features of human progeroid syndromes and age-related hematopoietic impairments. The study explores the effects of distinct and potentially synergistic interventions on platelet biology by incorporating both dietary restriction (DR) and myostatin/activin blockade (sActRIIB). Using validated antibodies, standardised immunohistochemical methods, and a well-structured comparison across multiple treatment groups contributes to methodological rigour and enhances interpretability.

However, the study also has limitations. The small sample size may limit statistical power to detect more subtle effects. Moreover, only surface expression of GPIbα and P-Selectin was examined; functional platelet assays such as aggregation or clotting time were not included. Finally, as the findings are based on a model of DNA repair deficiency, they may not be fully generalisable to normal ageing or other age-related conditions.

6. CONCLUSION

This study demonstrated that platelet dysfunction in DNA repair-deficient *Ercc1*$^{\Delta/-}$ mice is characterised by reduced GPIbα expression and unchanged P-Selectin levels compared to wild-type controls. While myostatin/activin blockade using sActRIIB modestly improved these markers—significantly reducing P-Selectin—dietary restriction alone or in combination had no significant effect. These findings suggest that targeting the myostatin/activin pathway may offer limited but specific benefits in modulating platelet activation in progeroid ageing, whereas dietary restriction alone appears insufficient. Overall, the results emphasise the need for more targeted interventions to restore platelet homeostasis in the context of genomic instability.

REFERENCES

[1] M. B. Birkisdóttir, I. van Galen, R. M. C. Brandt, S. Barnhoorn, N. van Vliet, C. van Dijk, B. Nagarajah, S. Imholz, C. T. van Oostrom, E. Reiling, Á. Gyenis, P. G. Mastroberardino, D. Jaarsma, H. van Steeg, J. H. J. Hoeijmakers, M. E. T. Dollé, and W. P. Vermeij, "The use of progeroid DNA repair-deficient mice for assessing anti-aging compounds, illustrating the benefits of nicotinamide riboside," *Front Aging,* vol. 3, p. 1005322, 2022. doi: 10.3389/fragi.2022.1005322

[2] S. Xia, M. Wang, X. Mo, J. Wang, S. Zheng, and X. Shen, "Moderate dietary restriction across generations promotes sustained health and extends lifespan by enhancing antioxidant capacity in Bombyx mori," *Scientific Reports,* vol. 15, no. 1, p. 17533, 2025/05/20 2025. doi: 10.1038/s41598-025-02528-4
[3] C. M. Gedik, G. Grant, P. C. Morrice, S. G. Wood, and A. R. Collins, "Effects of age and dietary restriction on oxidative DNA damage, antioxidant protection and DNA repair in rats," *Eur J Nutr,* vol. 44, no. 5, pp. 263-72, Aug 2005. doi: 10.1007/s00394-004-0520-0
[4] M. Chimen, A. Evryviadou, C. L. Box, M. J. Harrison, J. Hazeldine, L. H. Dib, S. J. Kuravi, H. Payne, J. M. J. Price, D. Kavanagh, A. J. Iqbal, S. Lax, N. Kalia, A. Brill, S. G. Thomas, A. Belli, N. Crombie, R. A. Adams, S. A. Evans, H. Deckmyn, J. M. Lord, P. Harrison, S. P. Watson, G. B. Nash, and G. E. Rainger, "Appropriation of GPIbα from platelet-derived extracellular vesicles supports monocyte recruitment in systemic inflammation," *Haematologica,* vol. 105, no. 5, pp. 1248-1261, May 2020. doi: 10.3324/haematol.2018.215145
[5] W. P. Vermeij, K. Alyodawi, I. van Galen, J. L. von der Heide, M. B. Birkisdóttir, L. J. Van't Sant, R. A. Ozinga, D. S. J. Komninos, K. Smit, Y. M. A. Rijksen, R. M. C. Brandt, S. Barnhoorn, D. Jaarsma, S. Vaiyapuri, O. Ritvos, T. B. Huber, O. Kretz, and K. Patel, "Improved health by combining dietary restriction and promoting muscle growth in DNA repair-deficient progeroid mice," *J Cachexia Sarcopenia Muscle,* vol. 15, no. 6, pp. 2361-2374, Dec 2024. doi: 10.1002/jcsm.13570
[6] R. W. Hart and A. Turturro, "Dietary restrictions and cancer," *Environmental Health Perspectives,* vol. 105, no. suppl 4, pp. 989-992, 1997/06/01 1997. doi: 10.1289/ehp.97105s4989
[7] K. S. Iyer and S. Dayal, "Modulators of platelet function in aging," *Platelets,* vol. 31, no. 4, pp. 474-482, 2020. doi: 10.1080/09537104.2019.1665641
[8] J. Peng, P. Friese, E. Heilmann, J. N. George, S. A. Burstein, and G. L. Dale, "Aged platelets have an impaired response to thrombin as quantitated by P-selectin expression," Blood, vol. 83, no. 1, pp. 161-166, 1994. doi: 10.1182/blood.V83.1.161.161
[9] R. Yan, Y. Xia, K. Zhou, J. Liu, Y. Sun, C. He, X. Ge, M. Yang, C. Sun, and L. Yuan, "Essential role of glycoprotein Ibα in platelet activation," *Blood Advances,* vol. 8, no. 13, pp. 3388-3401, 2024. doi: 10.1182/bloodadvances.2023012308
[10] M. K. Poddar and S. Banerjee, "Molecular Aspects of Pathophysiology of Platelet," *Platelets,* p. 21, 2020. doi: 10.5772/intechopen.92856
[11] R. P. Ertl, J. Chen, C. M. Astle, T. M. Duffy, and D. E. Harrison, "Effects of dietary restriction on hematopoietic stem-cell aging are genetically regulated," *Blood,* vol. 111, no. 3, pp. 1709-16, Feb 1 2008. doi: 10.1182/blood-2007-01-069807
[12] K. E. O'Connell, W. Guo, C. Serra, M. Beck, L. Wachtman, A. Hoggatt, D. Xia, C. Pearson, H. Knight, M. O'Connell, A. D. Miller, S. V. Westmoreland, and S. Bhasin, "The effects of an ActRIIb receptor Fc fusion protein ligand trap in juvenile simian immunodeficiency virus-infected rhesus macaques," *Faseb j,* vol. 29, no. 4, pp. 1165-75, Apr 2015. doi: 10.1096/fj.14-257543
[13] G. Weeda, I. Donker, J. de Wit, H. Morreau, R. Janssens, C. J. Vissers, A. Nigg, H. van Steeg, D. Bootsma, and J. H. Hoeijmakers, "Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence," *Curr Biol,* vol. 7, no. 6, pp. 427-39, Jun 1 1997. doi: 10.1016/s0960-9822(06)00190-4
[14] J. Chen, J. A. Schroeder, X. Luo, R. R. Montgomery, and Q. Shi, "The impact of GPIbα on platelet-targeted FVIII gene therapy in hemophilia A mice with pre-existing anti-FVIII immunity," *J Thromb Haemost,* vol. 17, no. 3, pp. 449-459, Mar 2019. doi: 10.1111/jth.14379
[15] L. Nicolai, R. Kaiser, R. Escaig, M. L. Hoffknecht, A. Anjum, A. Leunig, J. Pircher, A. Ehrlich, M. Lorenz, H. Ishikawa-Ankerhold, W. C. Aird, S. Massberg, and F. Gaertner, "Single platelet and megakaryocyte morpho-dynamics uncovered by multicolor reporter mouse strains in vitro and in vivo," *Haematologica,* vol. 107, no. 7, pp. 1669-1680, Jul 1 2022. doi: 10.3324/haematol.2021.278896
[16] J. Tsao, D. A. Vernet, R. Gelfand, I. Kovanecz, G. Nolazco, K. W. Bruhn, and N. F. Gonzalez-Cadavid, "Myostatin genetic inactivation inhibits myogenesis by muscle-derived stem cells in vitro but not when implanted in the mdx mouse muscle," *Stem Cell Res Ther,* vol. 4, no. 1, p. 4, Jan 7 2013. doi: 10.1186/scrt152
[17] I. Morianos, G. Papadopoulou, M. Semitekolou, and G. Xanthou, "Activin-A in the regulation of immunity in health and disease," *Journal of Autoimmunity,* vol. 104, p. 102314, 2019/11/01/ 2019. doi: https://doi.org/10.1016/j.jaut.2019.102314
[18] F. D. Bromer, A. Lodberg, M. Eijken, C. B. F. Andersen, M. F. Poulsen, J. S. Thomsen, and A. Brüel, "The Effect of Anti-Activin Receptor Type IIA and Type IIB Antibody on Muscle, Bone and Blood in Healthy and Osteosarcopenic Mice," *J Cachexia Sarcopenia Muscle,* vol. 16, no. 1, p. e13718, Feb 2025. doi: 10.1002/jcsm.13718
[19] W. P. Vermeij, M. E. Dollé, E. Reiling, D. Jaarsma, C. Payan-Gomez, C. R. Bombardieri, H. Wu, A. J. Roks, S. M. Botter, B. C. van der Eerden, S. A. Youssef, R. V. Kuiper, B. Nagarajah, C. T. van Oostrom, R. M. Brandt, S. Barnhoorn, S. Imholz, J. L. Pennings, A. de Bruin, Á. Gyenis, J. Pothof, J. Vijg, H. van Steeg, and J. H. Hoeijmakers, "Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice," *Nature,* vol. 537, no. 7620, pp. 427-431, Sep 15 2016. doi: 10.1038/nature19329

[20] A. T. Nurden and P. Nurden, "Inherited thrombocytopenias: history, advances and perspectives," *Haematologica,* vol. 105, no. 8, pp. 2004-2019, Aug 2020. doi: 10.3324/haematol.2019.233197
[21] N. G. Clavere, A. Alqallaf, K. A. Rostron, A. Parnell, R. Mitchell, K. Patel, and S. Y. Boateng, "Inhibition of activin A receptor signalling attenuates age-related pathological cardiac remodelling," *Dis Model Mech,* vol. 15, no. 5, May 1 2022. doi: 10.1242/dmm.049424
[22] M. Lebois, M. R. Dowling, P. Gangatirkar, P. D. Hodgkin, B. T. Kile, W. S. Alexander, and E. C. Josefsson, "Regulation of platelet lifespan in the presence and absence of thrombopoietin signaling," *J Thromb Haemost,* vol. 14, no. 9, pp. 1882-7, Sep 2016. doi: 10.1111/jth.13397
[23] R. Yan, Y. Xia, K. Zhou, J. Liu, Y. Sun, C. He, X. Ge, M. Yang, C. Sun, L. Yuan, S. Li, B. Yang, F. Meng, L. Cao, C. Ruan, and K. Dai, "Essential role of glycoprotein Ibα in platelet activation," *Blood Advances,* vol. 8, no. 13, pp. 3388-3401, 2024/07/09/ 2024. doi: https://doi.org/10.1182/bloodadvances.2023012308