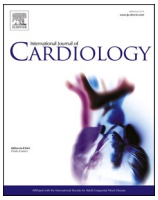
International Journal of Cardiology 442 (2026) 133861

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard



Stable Cytokine Network during Hypoxia and Exercise in Patients with Fontan Circulation☆

Julian Alexander von Hasselbach [a,b,*], Boris Dragutinovic [b], Nicole Müller [a], Johannes Breuer [a], Jens Tank [c], Jens Jordan [c,d], Wilhelm Bloch [b], Marijke Grau [b]

[a] Department of Pediatric Cardiology, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany
[b] Institute of Cardiology and Sports Medicine, Department of Molecular and Cellular Sports Medicine, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
[c] Institute of Aerospace Medicine, German Aerospace Centre (DLR), Linder Höhe, 51147 Cologne, Germany
[d] Medical Faculty, University of Cologne, 50931 Cologne, Germany

ARTICLE INFO

Keywords:
Fontan circulation
Hypoxia
Cytokines
Myokines
Inflammation

ABSTRACT

Background: Patients with Fontan circulation are often advised to avoid hypoxic exposure due to presumed cardiopulmonary vulnerability. Low-grade inflammation has also been reported in this population and may be influenced by hypoxia and/or exercise. Based on the potential interaction between hypoxia and submaximal exercise in modulating inflammatory signaling, we hypothesized that this combination could exacerbate subclinical inflammation.

Methods: Eighteen clinically stable patients with Fontan circulation (age 25 ± 6 years, 9 female, NYHA I) underwent a submaximal cycling step test under normoxia, followed by 24 hours of normobaric hypoxia ($FiO_2 \approx 15$ %, ~2500 m), and a second test under hypoxia at the :envihab research facility of the German Aerospace Centre. Venous blood was sampled at rest and peak exercise to analyse cytokines (IL-2, IL-6, TNF-α, TNF-β), adipomyokines (irisin, asprosin), and immune cell indices (WBC, lymphocytes, neutrophils, NLR, PLR, SII).

Results: Baseline cytokine levels and cell counts were within reference ranges. Exercise induced a mild increase in WBC under both conditions without affecting derived indices ($p \leq 0.05$). Cytokine concentrations remained stable. Irisin increased after normoxic ($p \leq 0.05$) but not hypoxic exercise; asprosin showed no significant changes.

Conclusion: Moderate normobaric hypoxia combined with submaximal exercise did not elicit systemic immune activation in stable patients with Fontan circulation. These data challenge assumptions of latent immune vulnerability and support the safety of controlled hypoxic exposure in the tested cohort.

1. Introduction

Patients with Fontan circulation are often advised to avoid hypoxic exposure, including air travel or high-altitude stays, due to potential cardiopulmonary risks [1]. The crosstalk between hypoxia and inflammatory signaling could exacerbate chronic low-grade inflammation [2], which has been linked with long-term complications like endothelial dysfunction, lymphatic malformations and organ impairment in this population [3,4]. Physical exercise can also alter immune activity, attributable to both cellular and cytokine responses, which may include immune cell counts and increased levels of inflammation-inducing cytokines [5,6]. Hypoxic exercise might thereby impose additional stresses, potentially unmasking immunologic dysregulation, latent inflammatory processes, or impaired adaptive responses.

However, evidence on immune responses to hypoxia and/or hypoxic exercise in patients with Fontan circulation is scarce. This study aimed to assess whether moderate normobaric hypoxia combined with submaximal exercise triggers systemic immune responses in clinically stable

☆ These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author at: Institute of Cardiology and Sports Medicine, Department of Molecular and Cellular Sports Medicine, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany.

E-mail addresses: J.vHasselbach@paed-sportmedizin.de (J.A. von Hasselbach), b.dragutinovic@dshs-koeln.de (B. Dragutinovic), nicole.mueller@ukbonn.de (N. Müller), johannes.breuer@ukbonn.de (J. Breuer), jens.tank@dlr.de (J. Tank), jens.jordan@dlr.de (J. Jordan), w.bloch@dshs-koeln.de (W. Bloch), m.grau@dshs-koeln.de (M. Grau).

https://doi.org/10.1016/j.ijcard.2025.133861
Received 21 August 2025; Accepted 2 September 2025
Available online 6 September 2025

patients with Fontan circulation. We analysed circulating immune cytokines, adipo-myokines, and immune cell markers under normoxic and hypoxic exercise following a controlled 24-hour normobaric hypoxic exposure.

2. Methods

We included 18 clinically stable patients with Fontan circulation (age 25 ± 6 years; 9 female, NYHA I), who were recruited from various heart centres across Germany and consented to participate in the study during the period from April to June 2022. Of those, 10 had a left and 8 a right systemic ventricle. Patients with resting oxygen saturation ≤ 90 % on room air, Fontan fenestration, pacemaker, nicotine abuse, pregnancy, pulmonary artery stents, or current use of pulmonary vasodilators such as sildenafil or bosentan were excluded.

At the :envihab research facility of the German Aerospace Centre (DLR, Cologne, Germany), participants initially performed a submaximal incremental step test on a bicycle ergometer (SANA comfort 150 S, Ergosana, Bitz, Germany), starting at 0.25 W/kg and increasing by 0.25 W/kg every three minutes, until reaching 1.25 W/kg under normoxic conditions. This was followed by a 24-hour normobaric hypoxic exposure ($FiO_2 \approx 15$ %), including an overnight stay simulating an altitude of approximately 2500 m above sea level (masl). Subsequently, a second submaximal exercise test was conducted under hypoxic conditions. To ensure patient safety, continuous electrocardiographic monitoring (Padsy Ergospiro, Medset Medizintechnik GmbH, Hamburg, Germany) and peripheral oxygen saturation (SpO_2) measurements (IntelliVueX3, Philips, Amsterdam, Netherlands) were conducted throughout both normoxic and hypoxic phases. Blood samples were collected at rest and at peak exercise under both normoxic and hypoxic conditions. Analysed cytokines included Interleukin (IL)-2, IL-6, Tumor necrosis factor (TNF) α and TNF-β. Adipo-myokines irisin and asprosin were assessed, as well as immune cell markers that included platelets, leukocytes, lymphocytes, and neutrophils. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) were determined according to previously published protocols [5]. In addition, the systemic immune-inflammation index (SII) was calculated according to the formula:

$$SII = Neutrophil\ Counts\ (10^3/\mu l)\ x\ (Platelet\ Counts\ (10^3/\mu l)\ x\ Lymphocyte\ Counts\ (10^3/\mu l)^{-1})$$

All cytokines were assessed using EDTA plasma by commercially available enzyme-linked immunosorbent assays (ELISA), adhering strictly to the manufacturers' instructions. Plasma irisin concentrations were determined using the Human Irisin ELISA (EK-067-29, Phoenix Pharmaceuticals, Burlingame, CA, USA). IL-2 (D2050), IL-6 (D6050), IL-10 (D1000B), and TNF-α (DTA00D) were analysed using ELISAs from Bio-Techne (Minneapolis, MN, USA). Asprosin levels were measured using Abcam's ELISA (ab275108, Abcam, Cambridge, UK). TNF-β concentrations were determined with the Human TNF-β ELISA (Thermo Fisher Scientific, Waltham, MA, USA).

The study was registered in the German Clinical Trials Register (DRKS) under the indentifier DRKS00025989 (https://www.drks.de). The study protocol was approved by the local ethics committee of the University of Bonn (application number 054/20) and the ethics committee of the North Rhine Medical Association (application number 2020046). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants and, if applicable, their legal guardians prior to enrolment.

2.1. Statistical analysis

Continuous variables such as cytokine concentrations, adipo-myokine levels, and immune cell counts were summarized using mean ± standard deviation. For each parameter, only participants with complete data across all time points were included in the analysis. Statistical analysis used a two-way mixed-effects ANOVA (within-subject factor: time; between-subject factor: condition). Participants with individual data points identified as extreme outliers were excluded from further analysis for the respective parameter. The significance level was defined as $p \leq 0.05$.

3. Results

SpO_2 at the time of blood sampling was 94 ± 3 % at rest in normoxia and 87 ± 3 % at rest in hypoxia, declining at peak exercise to 90 ± 4 % (normoxia) and 80 ± 6 % (hypoxia), respectively.

Baseline cytokine concentrations were as follows: IL-2 1.66 ± 2.14 pg/ml, IL-6 3.4 ± 3.2 pg/ml, TNF-α 4.95 ± 3.04 pg/ml and TNF-β 320.1 ± 210.2 pg/ml. Cytokine levels remained stable during exercise in both normoxia and hypoxia (Fig. $1_{a\text{-}d}$).

Immune cell counts, including lymphocytes and neutrophils, remained unchanged throughout the study, while white blood cells (WBC) increased mildly but significantly with submaximal exercise under both conditions (Table 1). The inflammatory cell-based indices NLR, PLR and SII stayed stable.

Irisin baseline levels were 15.36 ± 5.27 ng/ml and increased significantly with normoxic exercise but not with hypoxic exercise. Asprosin baseline levels were 46.16 ± 3.06 ng/ml with no significant changes during exercise or hypoxia.

4. Discussion

To our knowledge, this is the first study to investigate the combined effects of prolonged hypoxia and submaximal exercise in patients with Fontan circulation. These findings provide additional evidence to guide the assessment of short- to medium-term risks associated with hypoxic exposure in this cohort.

Baseline cytokine concentrations were within established normal ranges [6,7], indicating no chronic systemic immune activation in this cohort as previously described [3]. Cytokine levels for IL-2, IL-6, TNF-α and TNF-β remained unchanged during exercise in both normoxia and hypoxia, possibly reflecting insufficient exercise intensity or hypoxic dose to elicit responses, as increases are typically observed after strenuous exercise or higher altitude exposure [8–10].

White blood cells (WBC) remained within physiological range throughout the intervention. Submaximal exercise under both conditions led to a mild but significant WBC increase, consistent with previous findings and belongs to a multifactorial system including neuro-endocrinal factors, which was shown to be dose- and intensity-dependent [8]. However, the effects of hypoxia on WBC counts remain inconclusive, with studies reporting either no change [11] or increased cell numbers [12]. Thus, additional inflammatory cell-based indices—including NLR, PLR, and SII— were measured. Although mean values of NLR tended to be higher than reported averages from healthy controls, they remained within the 95 % reference range also during hypoxia exposure [13]. As these markers are recognized predictors of adverse outcomes in various clinical conditions, and higher values have been linked to Fontan-related complications (e.g., lymphatic malformations, protein-losing enteropathy) [4,5,14], their stability taken together with stable physiologic WBC counts in our cohort suggests that 24-hour hypoxia exposure does not induce a relevant cellular immune activation in patients with Fontan circulation.

Irisin, an adipo-myokine, showed physiological baseline values and increased significantly with exercise in normoxia, but not hypoxia, aligning with previous studies demonstrating hypoxia-related suppression possibly via HIF-1α mediated regulation, which may cause muscle atrophy and weakness [15,16]. Asprosin showed slightly elevated baseline levels but no significant changes with hypoxia or exercise, indicating limited acute responsiveness in this context, although its precise immunomodulatory role requires further investigation.

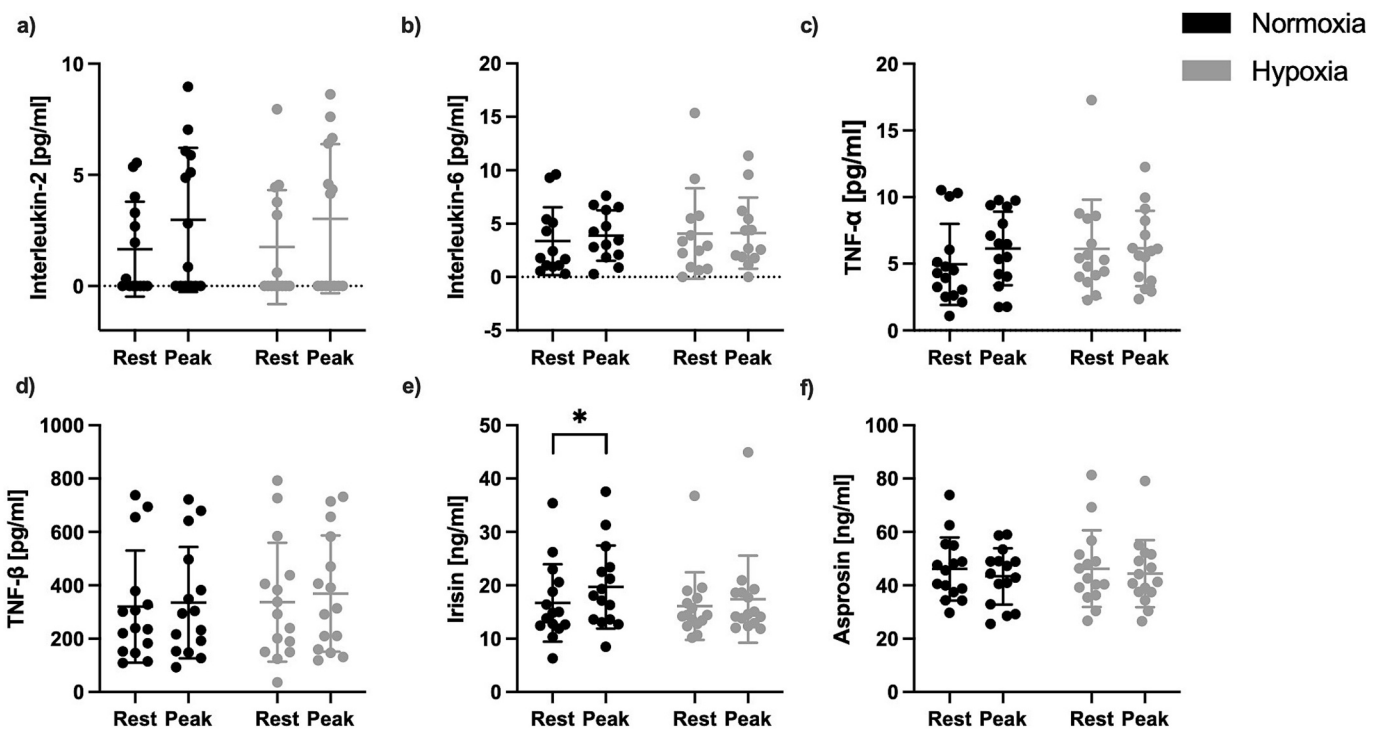


Fig. 1. Cytokines/Myokines measured in blood plasma at rest and peak exercise in normoxia and hypoxia.
Significant effects were demonstrated only for Irisin. n = number of included values.
a) IL-2 ($n = 14$); b) IL-6 ($n = 13$); c) TNF-α ($n = 15$); d) TNF-β ($n = 15$); e) Irisin ($n = 14$) significantly increased at peak exercise in normoxia, but no change was observed for values in hypoxia; f) Asprosin ($n = 15$); $^*p \leq 0.05$.

Table 1
Immune cell parameters at rest and at peak workload in normoxia and hypoxia.

		Rest		Peak					
		Normoxia	Hypoxia	Normoxia	Hypoxia	Group rest	Group peak	Time n	Time h
						(p-value)	(p-value)	(p-value)	(p-value)
Platelets ($n = 12$)	G/l	209 ± 59.9	187 ± 60.1			**0.04***			
White blood cells	G/l	6.8 ± 1.9	7.1 ± 1.5	8.7 ± 2.4	8.4 ± 2.5	0.54	0.42	**<0.001*****	**0.008***
Lymphocytes ($n = 16$)	G/l	2.1 ± 0.7	2.2 ± 0.6	2.6 ± 0.9	2.6 ± 0.9	0.54	0.71	**0.008***	**0.014***
Neutrocytes ($n = 14$)	G/l	4.1 ± 1.1	4.5 ± 1.2	5.1 ± 1.7	5.1 ± 1.8	0.23	0.29	**0.008***	0.38
NLR ($n = 14$)		2.1 ± 0.7	2.2 ± 0.8	2.1 ± 0.7	2.1 ± 0.7	0.46	0.33	0.68	0.29
PLR ($n = 12$)		127.1 ± 31.3	118.4 ± 40.6			0.23			
SII ($n = 12$)	G/l	535.2 ± 223.4	526.4 ± 233			0.87			

Group: p-value for comparison between normoxia and hypoxia at rest (Group rest) and at peak exercise (Group peak). Time: p-value for the comparison of exercise in normoxia (Time n) and in normobaric hypoxia (Time h).

5. Conclusion

In conclusion, clinically stable patients with Fontan circulation demonstrated immunological resilience to 24-hour exposure at 2500 masl and submaximal exercise, with no evidence of pathological systemic immune activation. These findings challenge assumptions of relevant latent immune vulnerability in the tested cohort and support the safety of controlled hypoxic and physical stress, potentially informing risk assessment for safe travel and physical activity planning in this group.

6. Study limitations

The study included only 18 clinically stable patients, selected using strict inclusion criteria, which may limit the generalizability of the findings to the broader Fontan population.

The hypoxic exposure was moderate ($FiO_2 \approx 15$ %, ~2500 m), more severe hypoxia or longer exposure might elicit different responses. Additionally, differences between physiological responses to normobaric and hypobaric hypoxia have been described; therefore, a different immune response under hypobaric conditions cannot be excluded. However, normobaric hypoxia is commonly used as a model to study hypoxia adaptations in clinical and experimental settings and provides a good approximation to real-life conditions.

Submaximal exercise may not reflect effects of high-intensity workloads. However, submaximal exertion is more representative of physical activities that these patients typically perform, such as those encountered during recreational mountain exposure.

CRediT authorship contribution statement

Julian Alexander von Hasselbach: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. **Boris Dragutinovic:** Data curation, Formal analysis, Investigation, Software, Writing – review & editing. **Nicole Müller:**

Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review & editing. **Johannes Breuer:** Resources, Supervision, Validation, Writing – review & editing. **Jens Tank:** Investigation, Supervision, Resources, Validation, Writing – review & editing. **Jens Jordan:** Resources, Supervision, Validation, Writing – review & editing. **Wilhelm Bloch:** Resources, Supervision, Validation, Writing – review & editing. **Marijke Grau:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

The authors express their gratitude to Anika Voß for her valuable technical support. They also sincerely thank the study participants and their families for their contribution and commitment to the study.

Acknowledgement of grant support: This study was supported by Stiftung KinderHerz Deutschland gGmbH (grant number 2511-5f-20-005). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

[1] N. Muller, J.A. Hartel, J. Schmitz, U. Baur, M. von der Wiesche, I. Rieger, et al., Peripheral oxygenation and pulmonary hemodynamics in individuals with Fontan circulation during 24-hour high-altitude exposure simulation, Circulation 149 (18) (2024) 1466–1468.

[2] H.K. Eltzschig, P. Carmeliet, Hypoxia and inflammation, N. Engl. J. Med. 364 (7) (2011) 656–665.

[3] A. Saraf, C. De Staercke, I. Everitt, A. Haouzi, Y.A. Ko, S. Jennings, et al., Biomarker profile in stable Fontan patients, Int. J. Cardiol. 305 (2020) 56–62.

[4] J. Moosmann, C. Schroeder, R. Cesnjevar, K. Rottermann, A. Weigelt, S. Dittrich, Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in Univentricular patients from birth to follow-up after Fontan-predicting lymphatic abnormalities, Front. Pediatr. 9 (2021) 740951.

[5] P. Wahl, S. Mathes, W. Bloch, P. Zimmer, Acute impact of recovery on the restoration of cellular immunological homeostasis, Int. J. Sports Med. 41 (1) (2020) 12–20.

[6] E.A. Said, I. Al-Reesi, N. Al-Shizawi, S. Jaju, M.S. Al-Balushi, C.Y. Koh, et al., Defining IL-6 levels in healthy individuals: a meta-analysis, J. Med. Virol. 93 (6) (2021) 3915–3924.

[7] A.A. Gharamti, O. Samara, A. Monzon, G. Montalbano, S. Scherger, K. DeSanto, et al., Proinflammatory cytokines levels in sepsis and healthy volunteers, and tumor necrosis factor-alpha associated sepsis mortality: a systematic review and meta-analysis, Cytokine 158 (2022) 156006.

[8] B.K. Pedersen, A.D. Toft, Effects of exercise on lymphocytes and cytokines, Br. J. Sports Med. 34 (4) (2000) 246–251.

[9] G. Hartmann, M. Tschop, R. Fischer, C. Bidlingmaier, R. Riepl, K. Tschop, et al., High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein, Cytokine 12 (3) (2000) 246–252.

[10] J. Lundeberg, J.R. Feiner, A. Schober, J.W. Sall, H. Eilers, P.E. Bickler, Increased cytokines at high altitude: lack of effect of ibuprofen on Acute Mountain sickness, physiological variables, or cytokine levels, High Alt. Med. Biol. 19 (3) (2018) 249–258.

[11] J.P. Hannon, J.L. Shields, C.W. Harris, Effects of altitude acclimatization on blood composition of women, J. Appl. Physiol. 26 (5) (1969) 540–547.

[12] I. Simon-Schnass, L. Korniszewski, The influence of vitamin E on rheological parameters in high altitude mountaineers, Int. J. Vitam. Nutr. Res. 60 (1) (1990) 26–34.

[13] L. Wu, S. Zou, C. Wang, X. Tan, M. Yu, Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in Chinese Han population from Chaoshan region in South China, BMC Cardiovasc. Disord. 19 (1) (2019) 125.

[14] S. Lattanzi, F. Brigo, E. Trinka, C. Cagnetti, M. Di Napoli, M. Silvestrini, Neutrophil-to-lymphocyte ratio in acute cerebral hemorrhage: a system review, Transl. Stroke Res. 10 (2) (2019) 137–145.

[15] S.S. Daskalopoulou, A.B. Cooke, Y.H. Gomez, A.F. Mutter, A. Filippaios, E. T. Mesfum, et al., Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects, Eur. J. Endocrinol. 171 (3) (2014) 343–352.

[16] S. Liu, P. Fu, K. Ning, R. Wang, B. Yang, J. Chen, et al., HIF-1alpha negatively regulates Irisin expression which involves in muscle atrophy induced by hypoxia, Int. J. Mol. Sci. 23 (2) (2022).