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Opinion paper

The Sickle Cell Pro-Inflammatory Response to Interval Testing Study (SPRINTS) in children and young adults with sickle cell anemia – Study design and methodological strategies

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ABSTRACT

The impact of sickle cell anemia (SCA) and its complications on physical functioning and cardiopulmonary/aerobic fitness in affected individuals is significant. Although limited data support the safety of maximal cardiopulmonary exercise testing (CPET) for children and adults with SCA, the safety of submaximal moderate and high intensity, and longer duration, exercise in this population is not clear. The Sickle Cell Pro-Inflammatory Response to Interval Testing Study (SPRINTS) is a multicenter, randomized, prospective trial. SPRINTS leverages unique collaborations between investigators in pediatric hematology and exercise science to evaluate the impact of exercise intensity on the acute phase inflammatory response to exercise and changes in airway dynamics in children and young adults with SCA. Here we describe the study design and methodological strategies employed in SPRINTS, including an exercise challenge that mimics real-life patterns of childhood physical activity, characterized by multiple moderate and high intensity brief bouts of exercise interspersed with rest periods. Primary outcomes comprise pre- and post-exercise biomarkers of inflammation and endothelial dysfunction and spirometry. Secondary outcomes include assessment of physical activity and functioning, genomic studies and near-infrared spectroscopy measurements to assess tissue oxygenation status during exercise. SPRINTS aims to enroll 70 subjects with SCA and 70 matched, healthy controls. We anticipate that data from SPRINTS will address gaps in our understanding of exercise responses and safety in SCA and support the future development of evidence-based, exercise prescription guidelines in this population.

1. Introduction

Acute and chronic complications of sickle cell anemia (SCA) have a major impact on the physical functioning of affected individuals. Children and adults with SCA suffer from complications such as chronic hemolytic anemia, debilitating acute and chronic pain, and end-organ damage, including cardiopulmonary disease. Combined, these complications reduce exercise ability and overall cardiopulmonary fitness [1–4]. Regular exercise and physical activity play an essential role in maintaining normal growth, development and well-being of children across the lifespan. However, concerns among providers, parents, and educators about disease-related physiological limitations represent a major barrier to physical activity and regular exercise for children with

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Abbreviations

CPET	Cardiopulmonary exercise test
CIIT	Controlled-intensity interval testing
DEXA	Dual energy x-ray absorptiometry
EIB	Exercise-induced bronchoconstriction
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 s
ICAM	Intercellular adhesion molecule
IL-6	Interleukin-6
NIRS	Near-infrared spectroscopy
PE-CAM	Platelet endothelial cell adhesion molecule
PFT	Pulmonary function test
SCA	Sickle cell anemia
sVCAM	Soluble vascular cell adhesion molecule
VO_2	Oxygen consumption
WBC	White blood cell

chronic medical conditions [5]. Despite treatment advances that have improved overall outcomes for children and adults with SCA, exercise prescription guidelines and recommendations do not exist for this population.

The ability to identify the optimal dose of exercise, while recognizing the specific risks and benefits for children with SCA, remains a challenge. As chronic and acute inflammation and endothelial dysfunction are major contributors to disease pathophysiology and complications in SCA, moderate to vigorous physical activity and exercise are often avoided in the SCA population due to concerns that the well-described inflammatory effects of exercise may precipitate complications such as sickle cell pain or exercise-induced bronchoconstriction (EIB) [6–8]. The current literature has major gaps in guiding parents, clinicians, or physical education teachers and coaches on how to balance the well-known benefits of physical activity and exercise against the potential harms in a disease like SCA. The Sickle Cell Pro-Inflammatory Response to Interval Testing Study (SPRINTS) was designed with this in mind and seeks to determine the safety margin for moderate to vigorous physical activity and exercise in children and young adults with SCA.

SPRINTS is a multicenter, randomized, prospective trial funded by the National Heart, Lung, and Blood Institute that aims to determine the impact of exercise intensity on: 1) the acute phase inflammatory response to exercise; and 2) changes in airway dynamics (i.e. airway bronchoconstriction) by spirometry. The study tests the hypotheses that: 1) compared to moderate exercise, vigorous exercise challenge is not associated with any greater acute increase in soluble vascular cell adhesion molecule (sVCAM) and other biomarkers of inflammation and endothelial dysfunction in children and young adults with SCA; and 2) the risk of EIB is greater in children and young adults with SCA compared to controls. SPRINTS also represents a multidisciplinary collaboration among investigators with expertise in the fields of SCA, exercise science and exercise immunology. In this paper, we describe the study design and methodological strategies that are employed in SPRINTS, including unique aspects of cardiopulmonary exercise testing and fitness assessment.

2. Material and methods

2.1. Study design, planned enrollment, and eligibility

SPRINTS is currently being conducted at Ann & Robert H. Lurie Children's Hospital of Chicago and the University of Illinois at Chicago, both in Chicago, IL, and St. Jude Children's Research Hospital in Memphis, TN. SPRINTS is registered on ClinicalTrials.gov (NCT03653676). IRB approval of the SPRINTS protocol was obtained at each participating site prior to subject recruitment.

Investigators at each site include a pediatric hematologist with expertise in SCA and an exercise physiologist or expert in exercise testing. The study aims to enroll 70 subjects with SCA and 70 matched, healthy controls without sickle cell trait. Following screening for eligibility, participants undergo a standard maximal cardiopulmonary exercise test (CPET) on a cycle ergometer. Participants subsequently undergo a controlled-intensity interval testing (CIIT) exercise challenge randomized to either a moderate intensity or vigorous intensity arm, as determined by the maximal workload achieved on initial CPET (Fig. 1). The randomization schema allows for the direct evaluation of exercise intensity on study outcomes in children and young adults with SCA given that the safety profile of moderate versus vigorous exercise is not well understood in this population.

Inclusion criteria for subjects include: 1) Male or female sex; 2) 10–21 years old; and 3) Hemoglobin SS or S/β^0 thalassemia genotype. Exclusion criteria include: 1) Inability to perform CPET due to physical limitation (e.g. severe hip osteonecrosis or stroke); 2) Enrollment in chronic transfusion program; 3) History of exercise-induced arrhythmia or syncope; 4) Diagnosis of asthma, defined as physician diagnosis plus use of asthma medications (daily controller or intermittent rescue), or by positive screening on the American Thoracic Society Division of Lung Diseases (ATS-DLD-78-C) questionnaire; 4) Known EIB, defined as physician diagnosis by exercise challenge test; 5) History of any cardiac diagnosis precluding exercise testing, unless cleared by a cardiologist; or 6) Any other condition that would place the individual at increased risk from study procedures or preclude the individual's full compliance with or completion of the study. All participants provide consent (if 18 years or older) or parental consent and child assent (if under age 18) prior to study participation.

2.2. Exercise testing and challenge

At each site, all subjects and controls undergo maximal CPET with breath-by-breath gas exchange utilizing a standard, modified Godfrey protocol with an electronically braked cycle ergometer [9]. Initial workload and workload increments are based on subject height: 8 W for <120 cm, 10 W for 120–150 cm, and 15 W for >150 cm. Subsequent increments occur at 1-min intervals via a continuous ramping protocol until volitional exhaustion. Continuous monitoring during exercise includes: 1) breath-by-breath, gas exchange analysis via indirect calorimetry; 2) 12-lead electrocardiogram; 3) regular blood pressure assessment; and 4) continuous pulse oximetry.

The CIIT protocol, adapted from published protocols, consists of 8 acute bouts of constant workload cycling with a rest interval of no cycling between each bout of exercise [10] (Fig. 2). Workload during exercise bouts is calculated individually for every subject or control equivalent to approximately 50% (moderate intensity arm) or 70% (vigorous intensity arm) of the peak workload achieved during the maximal CPET. The 8 exercise bouts for both the moderate and vigorous intensity CIIT arms are 2 min each in duration with 1-min rest intervals between bouts for a total CIIT duration of 24 min (i.e. total exercise time of 16 min). The protocol also includes a 1-min warm-up at the beginning and 5 min of unloaded recovery cycling at the end of the challenge. This exercise challenge was chosen because, compared to maximal CPET, multiple brief bouts of exercise more closely resemble real-life play and exercise patterns in children. Children rarely engage in exercise of maximal intensity and/or a prolonged duration. Instead, their play and sports participation consist of short bursts of moderate to high-intensity activities divided by intervals of low-intensity activities.

2.3. Other procedures and biomarker collection

All participants undergo baseline pulmonary function testing (PFT), including full spirometry, total body plethysmography for lung volumes,

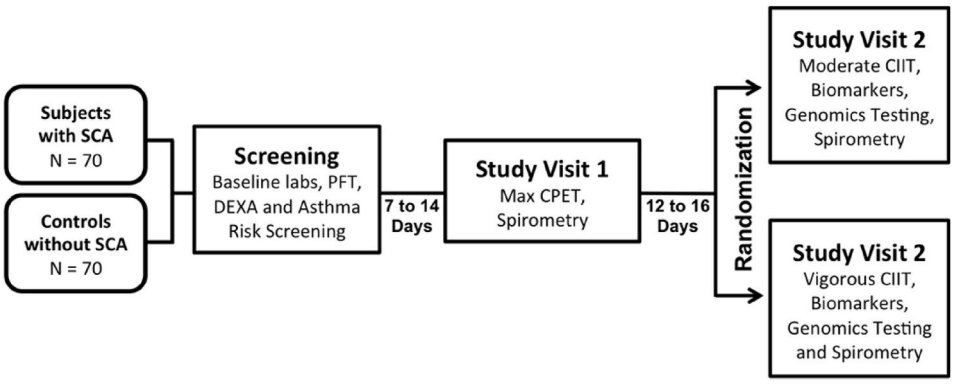

Fig. 1. Study schema for SPRINTS. Participants undergo screening and Visit 1, followed by randomization for Visit 2. In some cases, screening and Visit 1 occurred on the same day.

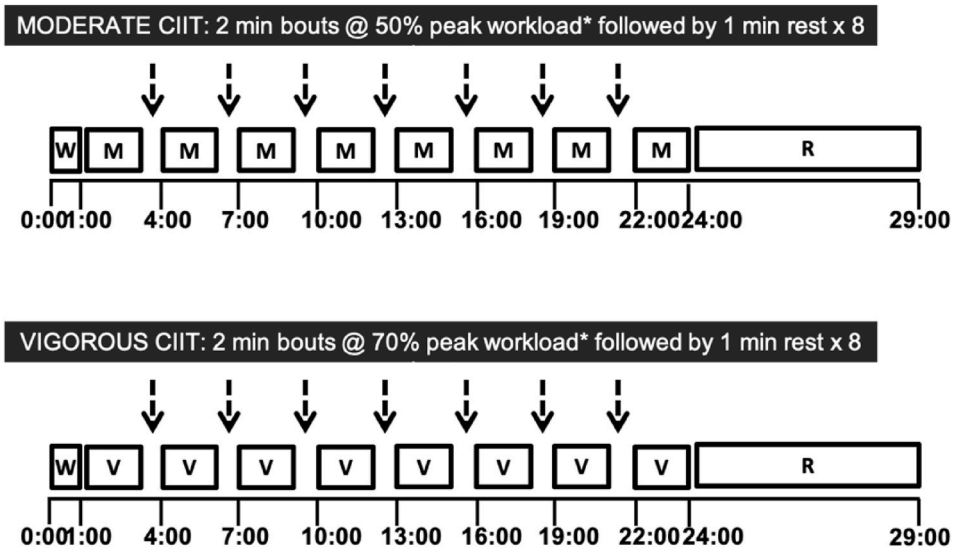

Fig. 2. Controlled-intensity interval testing (CIIT) protocol, which includes 8 bouts of cycling at either moderate or vigorous intensity with rest intervals in between cycling. Peak workload* refers to highest workload achieved during initial maximal CPET. (W, warm-up phase; M, moderate intensity exercise bout; V, vigorous intensity exercise bout; R, recovery phase).

and diffusion capacity measurements. Spirometry pre- and post-maximal CPET and CIIT exercise challenge is also performed. Other procedures include: 1) Baseline dual energy x-ray absorptiometry (DEXA) scan to determine body composition (i.e. % fat-free mass); and 2) Measurement of fractional exhaled nitric oxide (FeNO), a biomarker of airway inflammation, at baseline and after exercise testing. FeNO is obtained using the validated, point-of-care device, NIOX VERO® (Aerocrine, Inc.) [11].

Baseline laboratory studies are obtained through routine phlebotomy. Blood samples for biomarkers associated with the acute phase inflammatory response to exercise are collected before CIIT and after CIIT at times 0 and 60 min post-exercise. Pre- and post-CIIT samples are collected through a peripheral intravenous catheter, following a required 10-min waiting period after catheter insertion and before pre-CIIT sampling to reduce the potential impact of pain or discomfort on inflammatory biomarkers.

2.4. Study outcomes

The primary and secondary study outcomes in SPRINTS, along with their rationale, are listed in Table 1. The primary study outcomes focus on the systemic inflammatory response to exercise challenge and changes in spirometry associated with exercise that indicate potential EIB. The acute inflammatory response is defined by the changes in sVCAM and other adhesion molecules, including soluble intercellular adhesion molecule (ICAM), platelet endothelial cell adhesion molecule (PE-CAM), L-selectin, E-selectin, and P-selectin; interleukin-6 (IL-6); and total white blood cell (WBC) count and subsets from baseline to immediately after CIIT. These biomarkers were selected based on their known contribution to the underlying pro-inflammatory state and endothelial dysfunction in SCA, their association with SCA-related complications, and their potential upregulation during acute exercise [12–16]. The timing for spirometry evaluation before and after CIIT is adapted from American Thoracic Society (ATS) guidelines and recommendations for EIB, defined by measurement of forced expiratory volume in 1 s (FEV_1) pre-exercise and at 5, 10, 15, and 30 min post-exercise [17]. The concomitant requirement for post-exercise phlebotomy precludes obtaining FEV_1 measurements immediately after or 1 min after exercise. A comprehensive manual of operations was developed, and quality control measures were implemented to ensure accurate and reproducible primary data collection across sites (Table 2).

Secondary study outcomes are: 1) Questionnaires, including Patient-Reported Outcomes Measurement Information System (PROMIS®) physical functioning surveys, National Health and Nutrition Examination Survey (NHANES) physical activity questionnaire, and the International Study of Asthma and Allergies in Childhood (ISAAC) allergy

Table 1
Primary and secondary outcomes in SPRINTS.

	Outcome	Rationale or Justification
Primary Outcomes	Acute sVCAM response to exercise	• sVCAM is elevated at baseline and during complications in SCA
	Prevalence of EIB	• Lower airways disease (asthma and airway hyperreactivity) is common in SCA
Secondary Outcomes	Other biomarker responses (ICAM, PE-CAM, L-selectin, E-selectin, IL-6) to exercise	• Adhesion molecules and other biomarkers of inflammation and endothelial dysfunction are up-regulated in SCA • IL-6 is both a pro-inflammatory biomarker and a cytokine produced by muscle
	Safety events	• Safety of moderate to vigorous intensity exercise at longer durations is not clear in SCA
	Baseline pulmonary function test	• Abnormal findings on PFT are common in individuals with SCA and may affect fitness and cardiopulmonary responses to exercise
	DEXA scan	• Body composition and bone density influence cardiopulmonary fitness
	FeNO measurements	• FeNO is a biomarker of airway inflammation
	PROMIS® physical functioning surveys and NHANES physical activity questionnaires	• Self-reported physical functioning, mobility, and physical activity levels may impact cardiopulmonary fitness
	ISAAC questionnaire	• Symptoms suggestive of asthma and allergies may contribute to the understanding of lower airways disease and EIB in SCA
	Genomic studies (gene expression profiling)	• Gene expression patterns and profiling contribute to the understanding of molecular pathways that underlie benefits and risks of exercise
	Cerebral and quadriceps NIRS	• Muscle tissue oxygenation contributes to understanding of oxygen delivery during exercise

SCA, sickle cell anemia; sVCAM, soluble vascular cell adhesion molecule; EIB, exercise-induced bronchoconstriction; ICAM, intercellular adhesion molecule, PE-CAM, platelet endothelial cell adhesion molecule; IL-6, interleukin-6; PFT, pulmonary function test; DEXA, dual energy x-ray absorptiometry; FeNO, fractional exhaled nitric oxide; PROMIS®, Patient-Reported Outcomes Measurement Information System; NHANES, National Health and Nutrition Examination Survey; ISAAC; International Study of Asthma and Allergies in Childhood; NIRS, near-infrared spectroscopy.

Table 2
Quality control measures in SPRINTS.

Procedure	QC Measures Implemented across Sites
Exercise Testing	• Developed pre-exercise equipment checklist, including calibration requirements • Standardized data collection and data harmonization procedures across sites • Reviewed exercise data from single test control obtained in each exercise lab • Established central review of all gas exchange data
Spirometry	• Developed standardized instructions for baseline, pre- and post-exercise spirometry according to American Thoracic Society guidelines • Established central review of all spirometry data
Biomarker Collection	• Developed standardized instructions for baseline, pre- and post-exercise phlebotomy • Implemented standardized processing, storage and shipping of biomarker and genomic samples

screening questionnaire, administered to all participants at enrollment; 2), Pre- and post-CIIT exercise challenge sampling for genomic studies (i.e. microarray and gene expression profiling) [10]; and 3) Cerebral and quadriceps near-infrared spectroscopy (NIRS) evaluation during maximal CPET in a subset of participants at Lurie Children's Hospital [18].

2.5. Safety monitoring

The priority of evaluating safety outcomes in this study includes collection of all adverse and serious adverse events through phone calls to participants at 24 h and 1 week after exercise testing. For safety guidance during both the CPET and CIIT challenges, stopping rules were established to help determine whether or not participants could continue to exercise in the event they developed concerning symptoms or signs during exercise testing. A trial safety monitoring board was established and includes members not directly involved with the study with expertise in pediatric hematology, pediatric exercise medicine, pediatric pulmonary medicine, pediatric cardiology, and biostatistics. The board meets semi-annually to review study progress, protocol adherence, participant safety, data integrity, and data analysis and makes formal recommendations about study continuation to the principal and co-investigators.

2.6. Statistical considerations

The primary outcomes in SPRINTS are: 1) the acute sVCAM response in subjects with SCA undergoing moderate versus vigorous CIIT; and 2) the proportion of subjects versus controls diagnosed with EIB. Power calculation for sample size was based on a non-inferiority margin of 20% in the estimated difference in acute increase in sVCAM after moderate versus vigorous intensity exercise in subjects with SCA. The study considered a 20% between-group difference to be clinically significant based on data in the literature demonstrating this difference in sVCAM levels measured during vaso-occlusive pain versus acute chest syndrome [19], which may be reasonable to apply to the expected difference observed between exercise intensity arms in this trial. This sample size is also adequate to detect at least a 20% between-group difference in the proportion of patients with EIB with at least 80% power, the maximal between-group difference considered clinically significant from a safety standpoint. The number of subjects and controls in SPRINTS allows for attrition rates of up to 22% and 9% to ensure 80% power for the analysis of the acute sVCAM response and EIB, respectively.

For bivariate analyses, the acute sVCAM response will be compared in subjects with SCA undergoing moderate versus vigorous intensity CIIT. The prevalence of EIB will be compared in subjects versus controls by CIIT intensity. Sub-analyses for these primary outcomes will also be conducted through comparisons for all other participant combinations and biomarkers. A multivariable linear model will be developed to determine if the magnitude of the acute sVCAM response to exercise is independently associated with exercise intensity, with inclusion of participant type, sex, age, fitness (i.e. peak oxygen consumption, "VO_2"), total workload normalized for body mass, and hydroxyurea status as co-variables. A multivariable model will also be developed using either EIB status or FEV_1 change as a dependent variable and participant type, sex, age, exercise intensity, peak VO_2, total workload normalized for body mass, and hydroxyurea use as co-variables. Missing data will be reviewed and methods implemented to estimate or impute missing values.

3. Discussion

The SPRINTS trial was designed to address major gaps in knowledge about exercise safety for children and young adults with SCA. Current assumptions about exercise safety in SCA may be derived from published studies of maximal CPET, which suggest high-intensity exercise of short

duration associated with CPET challenge is feasible and largely safe in the SCA population [3,20–22]. Select published studies also suggest that exercise training for individuals with SCA is feasible and safe [23,24]. However, these intervention studies are limited by their small sample size and the submaximal effort required of the training sessions in the adult studies. The impact of longer duration, higher intensity exercise on safety outcomes, including adverse events, the acute inflammatory response, and airway hyperreactivity, remains unclear in SCA.

To address our study aims in SPRINTS, we employ a unique multidisciplinary, translational approach that bridges investigators in hematology with those in exercise science/medicine. Our reliance on investigators with expertise outside of hematology and sickle cell disease allowed us to design rigorous exercise protocols tailored to individuals with SCA and that will further our understanding of cardiopulmonary fitness and physical functioning in this population. The collaboration also required bi-directional learning and coordination of efforts between the disciplines to ensure seamless processes and workflows. Until recently, research focused on exercise and exercise training in individuals with SCA has been limited, relegating it behind other genetic and chronic conditions for which there is a clearer understanding of the relationship among fitness, physical activity, regular exercise and disease-related outcomes. In cystic fibrosis, for example, higher cardiopulmonary fitness and exercise training are associated with longitudinal improvements in lung function, cardiopulmonary fitness, and other patient-reported outcomes [25–28]. In SCA, a deeper understanding of patient-important outcomes such as physical functioning and fitness has become especially relevant due to improved lifespan of affected individuals and recent advances in disease-modifying therapeutics and curative options.

Another innovative aspect of SPRINTS is that we adopt the CIIT protocol as a novel paradigm for exercise testing. Although maximal CPET remains the gold standard for measuring cardiopulmonary fitness, logistical and theoretical concerns continue to arise about its utility in pediatric populations. Pushing individuals during exercise to volitional exhaustion, as required during maximal CPET testing, allows for evaluation of physiologic perturbations under maximal exercise stress. The effort and stress associated with incremental exercise to maximal exhaustion, however, are not representative of the usual physical activity patterns observed during play or exercise for most children. Children and adolescents generally exhibit short bursts of high intensity activity with interval rest periods during play time, exercise, and sports participation. The multiple, brief-bout CIIT exercise challenge adopted in SPRINTS has previously been shown to be feasible in the general pediatric population [29,30]. For this reason, we adapted this strategy for use in SPRINTS given that it both represents a more real-life pattern of exercise in children and allows for evaluation of exercise responses and safety under both moderate and vigorous exercise intensities.

The principal limitation in SPRINTS will be the potential for recruitment bias. Motivation and willingness to volunteer for a research study that requires blood draws and multiple study visits probably differs between children and young adults with and without SCA. Children and young adults with SCA, for example, have a lifetime familiarity with blood draws and medical settings, but most children and young adults from the control group would not. These differences could lead to selection of different segments of these populations of children and young adults. However, this limitation is less important for a safety trial and proof of concept study like SPRINTS than it would be for a study that aims to characterize exercise responses of an entire population.

We expect that the data derived from completing SPRINTS will advance our knowledge regarding the impact of exercise intensity on the acute phase exercise response in children and young adults with SCA. Simultaneous collection of safety data, including whether or not airway hyperreactivity is associated with exercise in a disease population predisposed to bronchoconstriction, will also be useful. We anticipate that the acute phase response to vigorous intensity exercise will not be any greater compared to that observed with moderate intensity exercise. However, we expect that the proportion of children and young adults demonstrating EIB could be greater in SCA due to the generally high prevalence of wheezing and asthma reported in these patients [31–33]. Together with our secondary data collection and planned ancillary studies, we will be uniquely positioned to begin to address some of the gaps in understanding the impact of exercise intensity on acute phase and lower airway responses in SCA. Moreover, these data will help inform the design of future intervention studies of exercise training for individuals with SCA. Ultimately, these results will contribute to the development of evidence-based exercise prescription guidelines in this population.

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Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

[1] R.J. Barst, K.K. Mubarak, R.F. Machado, K.I. Ataga, R.L. Benza, O. Castro, et al., Exercise capacity and haemodynamics in patients with sickle cell disease with pulmonary hypertension treated with bosentan: results of the ASSET studies, Br. J. Haematol. 149 (2010) 426–435.
[2] V.R. Gordeuk, C.P. Minniti, M. Nouraie, A.D. Campbell, S.R. Rana, L. Luchtman-Jones, et al., Elevated tricuspid regurgitation velocity and decline in exercise capacity over 22 months of follow up in children and adolescents with sickle cell anemia, Haematologica 96 (2011) 33–40.
[3] R.I. Liem, M. Reddy, S.A. Pelligra, A.P. Savant, B. Fernhall, M. Rodeghier, et al., Reduced fitness and abnormal cardiopulmonary responses to maximal exercise testing in children and young adults with sickle cell anemia, Physiological reports 3 (2015).
[4] V. Sachdev, G.J. Kato, J.S. Gibbs, R.J. Barst, R.F. Machado, M. Nouraie, et al., Echocardiographic markers of elevated pulmonary pressure and left ventricular diastolic dysfunction are associated with exercise intolerance in adults and adolescents with homozygous sickle cell anemia in the United States and United Kingdom, Circulation 124 (2011) 1452–1460.
[5] N. Shields, A.J. Synnot, An exploratory study of how sports and recreation industry personnel perceive the barriers and facilitators of physical activity in children with disability, Disabil. Rehabil. 36 (2014) 2080–2084.
[6] D. Zhang, C. Xu, D. Manwani, P.S. Frenette, Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology, Blood 127 (2016) 801–809.
[7] L. Belhassen, G. Pelle, S. Sediame, D. Bachir, C. Carville, C. Bucherer, et al., Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation, Blood 97 (2001) 1584–1589.
[8] Y.T. Shiu, M.M. Udden, L.V. McIntire, Perfusion with sickle erythrocytes up-regulates ICAM-1 and VCAM-1 gene expression in cultured human endothelial cells, Blood 95 (2000) 3232–3241.
[9] S. Godfrey, C.T. Davies, E. Wozniak, C.A. Barnes, Cardio-respiratory response to exercise in normal children, Clin. Sci. 40 (1971) 419–431.
[10] S. Radom-Aizik, F. Zaldivar, F. Haddad, D.M. Cooper, Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults, J. Appl. Physiol. 114 (2013) 628–636.
[11] S. Korn, M. Wilk, S. Voigt, S. Weber, T. Keller, R. Buhl, Measurement of fractional exhaled nitric oxide: comparison of three different analysers, Respiration; international review of thoracic diseases (2019) 1–8.
[12] N.M. Matsui, L. Borsig, S.D. Rosen, M. Yaghmai, A. Varki, S.H. Embury, P-selectin mediates the adhesion of sickle erythrocytes to the endothelium, Blood 98 (2001) 1955–1962.
[13] S.S. Signorelli, M.C. Mazzarino, L. Di Pino, G. Malaponte, C. Porto, G. Pennisi, et al., High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test, Vasc. Med. 8 (2003) 15–19.
[14] A.I. Bartzeliotou, A.P. Margeli, M. Tsironi, K. Skenderi, C. Bacoula, G.P. Chrousos, et al., Circulating levels of adhesion molecules and markers of endothelial activation in acute inflammation induced by prolonged brisk exercise, Clin. Biochem. 40 (2007) 765–770.
[15] E.S. Hatzipantelis, Z.D. Pana, N. Gombakis, A. Taparkou, V. Tzimouli, D. Kleta, et al., Endothelial activation and inflammation biomarkers in children and adolescents with sickle cell disease, Int. J. Hematol. 98 (2013) 158–163.
[16] A.J. Duits, R.C. Pieters, A.W. Saleh, E. van Rosmalen, H. Katerberg, K. Berend, et al., Enhanced levels of soluble VCAM-1 in sickle cell patients and their specific

increment during vasoocclusive crisis, Clin. Immunol. Immunopathol. 81 (1996) 96–98.
[17] J.P. Parsons, T.S. Hallstrand, J.G. Mastronarde, D.A. Kaminsky, K.W. Rundell, J. H. Hull, et al., An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction, Am. J. Respir. Crit. Care Med. 187 (2013) 1016–1027.
[18] G. Ganesan, S.Y. Leu, A. Cerussi, B. Tromberg, D.M. Cooper, P. Galassetti, Cerebral and muscle tissue oxygenation during incremental cycling in Male adolescents measured by time-resolved near-infrared spectroscopy, Pediatr. Exerc. Sci. 28 (2016) 275–285.
[19] V.S. Sakhalkar, S.P. Rao, J. Weedon, S.T. Miller, Elevated plasma sVCAM-1 levels in children with sickle cell disease: impact of chronic transfusion therapy, Am. J. Hematol. 76 (2004) 57–60.
[20] S. Ameringer, R.K. Elswick Jr., I. Sisler, W. Smith, T. Lipato, E.O. Acevedo, Exercise testing of adolescents and young adults with sickle cell disease: perceptual responses and the gas exchange threshold, J. Pediatr. Oncol. Nurs. 36 (2019) 310–320.
[21] L.A. Callahan, K.F. Woods, G.A. Mensah, L.T. Ramsey, P. Barbeau, B. Gutin, Cardiopulmonary responses to exercise in women with sickle cell anemia, Am. J. Respir. Crit. Care Med. 165 (2002) 1309–1316.
[22] E.J. van Beers, M.N. van der Plas, E. Nur, H.J. Bogaard, R.P. van Steenwijk, B. J. Biemond, et al., Exercise tolerance, lung function abnormalities, anemia, and cardiothoracic ratio in sickle cell patients, Am. J. Hematol. 89 (2014) 819–824.
[23] B. Gellen, L. Messonnier, A. Merlet, E. Audureau, T. Rupp, S. Peyrot, et al., Prospective controlled trial on endurance exercise training in adult sickle cell disease patients, Blood 130 (2017) 991.
[24] R.I. Liem, M. Akinosun, D.S. Muntz, A.A. Thompson, Feasibility and safety of home exercise training in children with sickle cell anemia, Pediatr. Blood Canc. 64 (2017).
[25] J. Bradley, F. Moran, Physical training for cystic fibrosis, Cochrane Database Syst. Rev. (2008). CD002768.
[26] J.M. Collaco, S.M. Blackman, K.S. Raraigh, C.B. Morrow, G.R. Cutting, S. M. Paranjape, Self-reported exercise and longitudinal outcomes in cystic fibrosis: a retrospective cohort study, BMC Pulm. Med. 14 (2014) 159.
[27] H. Hebestreit, S. Kieser, S. Junge, M. Ballmann, A. Hebestreit, C. Schindler, et al., Long-term effects of a partially supervised conditioning programme in cystic fibrosis, Eur. Respir. J. 35 (2010) 578–583.
[28] G.D. Wells, D.L. Wilkes, J.E. Schneiderman, S. Thompson, A.L. Coates, F. Ratjen, Physiological correlates of pulmonary function in children with cystic fibrosis, Pediatr. Pulmonol. 49 (2014) 878–884.
[29] S. Radom-Aizik, F. Zaldivar Jr., S.Y. Leu, D.M. Cooper, A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females, J. Appl. Physiol. 107 (2009) 168–175.
[30] S. Radom-Aizik, F. Zaldivar Jr., S.Y. Leu, D.M. Cooper, Brief bout of exercise alters gene expression in peripheral blood mononuclear cells of early- and late-pubertal males, Pediatr. Res. 65 (2009) 447–452.
[31] J.J. Field, J. Stocks, F.J. Kirkham, C.L. Rosen, D.J. Dietzen, T. Semon, et al., Airway hyperresponsiveness in children with sickle cell anemia, Chest 139 (2011) 563–568.
[32] N.A. Galadanci, W.H. Liang, A.A. Galadanci, M.H. Aliyu, B.W. Jibir, I.M. Karaye, et al., Wheezing is common in children with sickle cell disease when compared with controls, J. Pediatr. Hematol. Oncol. 37 (2015) 16–19.
[33] J.M. Knight-Madden, T.S. Forrester, N.A. Lewis, A. Greenough, Asthma in children with sickle cell disease and its association with acute chest syndrome, Thorax 60 (2005) 206–210.