Cardiology in the Young

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Subjective fitness relates to performance and can be improved by exercise in children and young adults with heart disease

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Original Article

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Abstract

Introduction: The Duke Activity Status Index is used to assess an individual patient's perception of their fitness abilities. It has been validated and shown to predict actual fitness in adults but has been studied less in the paediatric population, specifically those with heart disease. This study aims to assess if the Duke Activity Status Index is associated with measured markers of physical fitness in adolescents and young adults with heart disease. *Methods:* This retrospective single-centre cohort study includes patients who completed a minimum of 12 weeks of cardiac rehabilitation between 2016 and 2022. Cardiac rehabilitation outcomes included physical, performance, and psychosocial measures. A comparison between serial testing was performed using a paired *t*-test. Univariable and multivariable analyses for Duke Activity Status Index were performed. Data are reported as median [interquartile range]. *Results:* Of the 118 participants (20 years-old [13.9–22.5], 53% male), 33 (28%) completed at least 12 weeks of cardiac rehabilitation. Median peak oxygen consumption was 60.1% predicted [49–72.8%], and Duke Activity Status Index was 32.6 [21.5–48.8]. On Pearson's correlation assessing the Duke Activity Status Index, there were significant associations with % predicted peak oxygen consumption ($r = 0.49$, $p < 0.0001$), 6-minute walk distance ($r = 0.45$, $p < 0.0001$), Duke Activity Status Index metabolic equivalents ($r = 0.45$, $p < 0.0001$), and dominant hand grip ($r = 0.48$, $p < 0.0001$). In multivariable analysis, the % predicted peak oxygen consumption ($r = 0.40$, $p = 0.005$) and dominant hand grip ($r = 0.37$, $p = 0.005$) remained statistically significant. *Conclusions:* Duke Activity Status Index is associated with measures of physical fitness in paediatric and young adults with heart disease who complete a cardiac rehabilitation program.

Introduction

Cardiac rehabilitation is a validated clinical tool to improve fitness in those with heart disease. Fitness has been defined to include multiple facets including one's abilities, endurance, strength, and flexibility. In addition to directly measuring various physical fitness outcomes during cardiac rehabilitation, subjective patient questionnaires are often used to assess quality of life, emotional well-being, and perceptions of functional status in daily life. The questionnaires are used to evaluate emotional well-being because a comprehensive cardiac rehabilitation program focuses on both the physical and mental growth of the patient.[1] One of the questionnaires often used is the Duke Activity Status Index.

The Duke Activity Status Index has been shown to correlate significantly with functional status in adults and those with chronic medical conditions including chronic obstructive pulmonary disease and heart failure.[2,3,4] In addition to correlating with functional status, the Duke Activity Status Index has also been shown to predict health-related outcomes in the adult population following cardiac surgeries.[5] Minimal studies have been published showing the correlation of the Duke Activity Status Index in the paediatric population and specifically in children and young adults with heart disease including CHD. This is important because patients with CHD often have poor perceptions of their fitness.[6] Improving these perceptions of what activities they feel they can do, may result in a more positive mindset, potentially removing a barrier to the protective effects of exercise and fitness.[7]

This study aimed to; (1) assess the ability of the Duke Activity Status Index to correlate with functional measures of fitness in children and young adults with cardiac disease including CHD and (2) evaluate for improvements in the perceived (qualitative questionnaires) and actual fitness measures following completion of cardiac rehabilitation.

Materials and methods

This is a single-site retrospective cohort study assessing for perceived fitness abilities using the Duke Activity Status Index and comparing it to other markers of physical function for those completing cardiac rehabilitation for the first time between September 2016 to September 2022. Data on enrollment dates, number of completed weeks and sessions, and programme completion outcomes were collected. Additional data collected from the chart review included patients' demographic information and medical diagnoses. Exclusion criteria included missing Duke Activity Status Index data, no history of heart disease, and age less than 8 years old. If an individual completed multiple rounds of cardiac rehabilitation, the later encounters were excluded if the individual had previously completed at least 1 month of a cardiac rehabilitation program, or earlier encounters of less than 30 days were excluded if the individual completed more than 1 month in a cardiac rehabilitation program after that encounter.

Cardiac rehabilitation is a personalised exercise and lifestyle intervention program designed per the standard of the American Association of Cardiovascular and Pulmonary Rehabilitation and has been previously described.[8] Cardiac rehabilitation sessions took place at our hospital's cardiac rehabilitation facility. The programme included one-on-one supervision by a trained exercise physiologist. Age-appropriate activities were incorporated into sessions to increase motivation and enjoyment during cardiac rehabilitation.

Cardiac rehabilitation outcomes were recorded at the first and last cardiac rehabilitation sessions. These included physical body measurements, performance measures, and psychosocial questionnaires. Physical body measurements including height and weight were recorded. Cardiopulmonary exercise testing was performed on a stationary cycle ergometer (Corival; Lode; Groningen, The Netherlands) with an individualised ramp protocol using breath-by-breath analysis (Ultima CardiO2; MGC Diagnostics; Saint Paul, MN, USA), as previously described.[9] Criteria for a maximal effort exercise test were that 2 of the following 3 criteria were met: respiratory exchange ratio ≥1.10; maximal heart rate ≥85% of the age-predicted maximum (220-age in years); or maximal rating of perceived exertion >18 on a 6 to 20 Borg scale.[10] Predicted peak oxygen consumption was calculated per Wasserman et al. and Cooper et al. equations.[11,12]

Additional performance outcomes collected included the number of sit to stand repetitions within 30 s, sit and reach distance, the number of arm curls within 30 s with a constant weight, 6-minute walk test, and handgrip strength. Metabolic equivalents were calculated from the 6-minute walk distance[13]. A set of patient questionnaires were also collected at the first and last sessions.

The Duke Activity Status Index was designed to be a reliable tool to assess a patient's physical functional status.[14] The 12 questions are in a yes-no format and cover a wide range of activities. Scores are weighted based on the difficulty of the task in question with more difficult tasks having a higher score. Higher scores correlate with higher functional status, with a maximum score of 58.2 points.[14] Question #10 was removed for patients <18 years old as it asks about sexual activity, making a maximum score of 52.95 for patients <18 years old. Normative data for the Duke Activity Status Index does not exist for paediatric patients, but adult studies have shown prognostic significance when the score is less than 26–34.[15,16] Additionally, to account for absolute score differences between paediatric and adult patients, Duke Activity Status Index is also represented as a percentage of expected maximum points (i.e. Duke Activity Status Index % of total). Metabolic equivalents were calculated from the Duke Activity Status Index.[14]

Additional social and emotional surveys were provided and completed before and after cardiac rehabilitation including the Patient Health Questionnaire-9 screen for depression and the Short Form Health Inventory-36, which included the Mental Component Scoring (a combination of the vitality, social functioning, role-emotional, and mental health forms) and the Physical Component Scoring (a combination of the physical function, role-physical, bodily pain, and general health forms).[17,18]

Statistical analyses

Descriptive statistics are presented as median [interquartile range] for continuous variables and as n (frequency) for categorical variables. Baseline and final cardiac rehabilitation completion data were compared using a paired *t*-test. Groups were compared at baseline utilising the Student's *t*-test for independent groups with equal variances and Welch's *t*-test for unequal variances. All *t*-tests were two-sided with p value <0.05 considered significant. Univariable and multivariable analyses were then completed for the Duke Activity Status Index. Univariable analysis was performed with Pearson's correlation coefficient. The selection criteria for the multivariable model were a p value of 0.1 for entry and 0.05 to remain in the model. Candidate predictors for multivariable modelling were cardiac rehabilitation physical activity outcomes and included peak oxygen consumption, 6-minute walk distance, arm curls, sit to stand, and dominant handgrip. Statistical analyses were performed using JMP®, Version 16 from SAS Institute Inc. (Cary, NC). Figures were created with Microsoft Word, Excel, and PowerPoint (Redmond, WA).

Results

A total of 199 individual cardiac rehabilitation enrollments were evaluated during the study period (Figure 1). There were 81 enrollments (41%) excluded from this study: 38 for having completed more than 1 month of cardiac rehabilitation program previously, 13 enrollments that were less than 30 days of cardiac rehabilitation but the individual completed longer than 30 days later, 6 for individuals aged less than 8 years of age, 13 for individuals without underlying cardiac disease, and 11 for incomplete Duke Activity Status Index data. No patients included in the analysis had multiple rounds of cardiac rehabilitation. Of the 118 patients (aged 20 years old [13.9–22.5], 53% male) included in the analysis, 33 (28%) completed at least 12 weeks of the programme. There were no significant age or sex differences between those who did and did not complete cardiac rehabilitation. Full demographics for the study are included in Table 1. Of note, 67 of the 118 patients had pre-cardiac rehabilitation maximal effort cardiopulmonary exercise testing included. In the 33 patients who completed at least 12 weeks of the programme, 26 patients completed a maximal effort cardiopulmonary exercise test both before and after cardiac rehabilitation (Table 1).

Analysis for the whole cohort includes only data obtained from their pre-cardiac rehabilitation forms and testing as the majority of the cohort did not complete a full cardiac rehabilitation program (Table 1). The median Duke Activity Status Index was 32.6 [21.5–48.8] and the average median Duke metabolic equivalents was 3.4 [2.7–4.5]. There were 58% (69/118) of patients with a baseline

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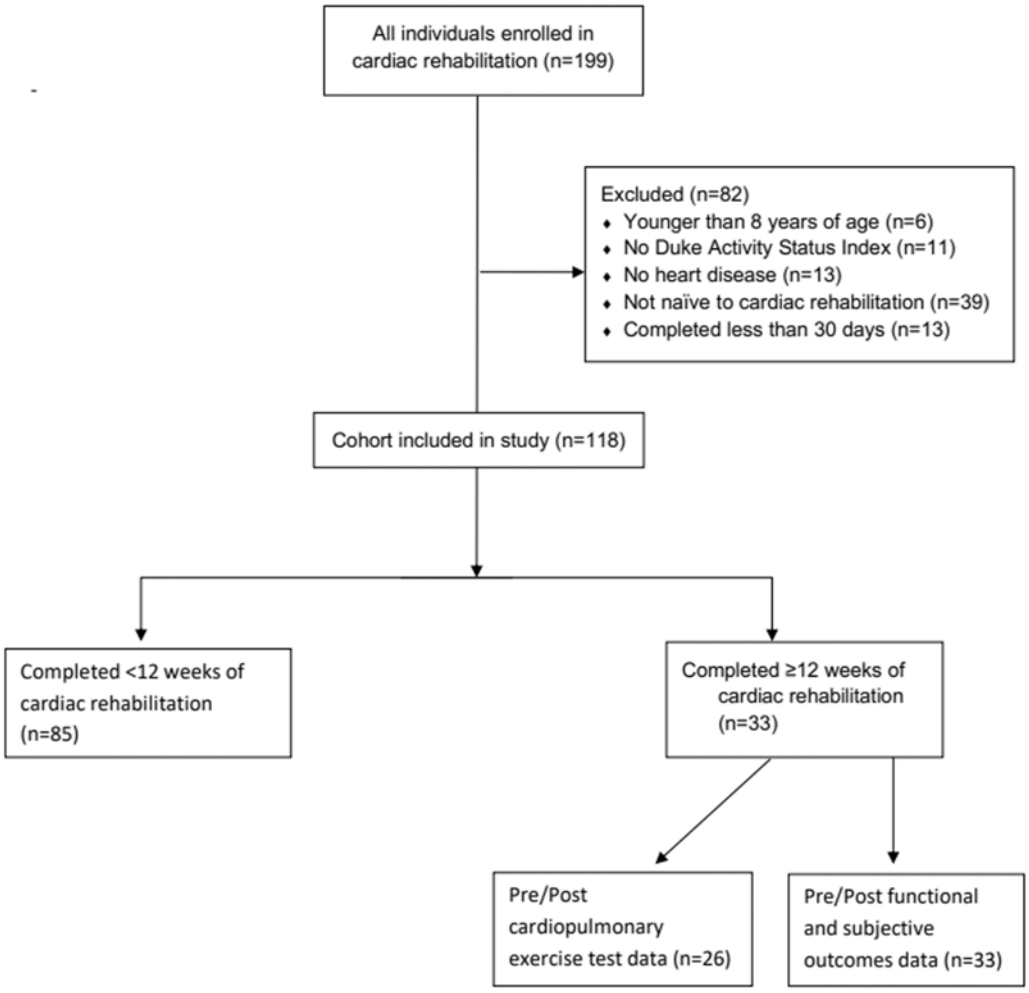

Figure 1. Flowsheet of inclusion of participants in the study. Not naïve to cardiac rehabilitation was defined as having completed at least 30 days of cardiac rehabilitation prior to starting a new cardiac rehabilitation session. For those who participated in programmes in which less than 30 days were completed, but a session of at least 30 days was completed later during the study period, then the prior sessions of less than 30 days were excluded as well. Of those included, those who completed at least 12 weeks of a cardiac rehabilitation program were assessed for completion of different components of the testing. The functional and subjective outcomes referred to in this flow chart include 6-minute walk test, functional strength assessments, and patient questionnaires.

Duke Activity Status Score <34 and 41% (48/118) with a Duke Activity Status Score <26. Median peak oxygen consumption was 60.1 [49–72.8] % of predicted, or 20.8 [16–24] ml/kg/min. The entire cohort trended towards having mild depression based on the median Patient Health Questionnaire-9 score of 5.5 [2–8.3].[17]

Among the subset who completed cardiac rehabilitation ($n = 33$), there was a statistically significant increase in Duke Activity Status Index (33.2 [24.2–46.7] versus 41.9 [31.5–53]; $p = 0.005$) and Duke Activity Status Index % of total (50.9 [45.0–84.1] versus 78.1 [54.3–100.0] %, $p = 0.0002$) (Supplemental Figure 1). In addition, there was a significant increase in patients who scored >34 (39% [13/33] versus 70% [23/33], $p = 0.01$), but there was not a significant increase in those who scored>26 (67 [22/33] versus 82% [27/33], $p = 0.2$) on the Duke Activity Status Index. The median change in Duke Activity Status Index was 11.2 [0–34.4]. On functional assessment, there was a significant increase in 6-minute walk distance (405.4 [310–490] versus 488.7 [383.5–563] m; $p < 0.0001$), dominant hand grip (11.2 [7.0–16.1] versus 12.9 [7.3–17.8] kg; $p = 0.006$), sit to stand repetitions (15.5 [12–19.5] versus 21.8 [17–27.5] repetitions; $p < 0.0001$), arm curls (20.1 [17–23.8] versus 25.1 [19.3–30] repetitions; $p < 0.0001$), and sit and reach distance (38.1 [29.2–43.2] versus 42.9 [33.850.8] cm; $p = 0.005$). Though most data from the cardiopulmonary exercise testing suggested a trend towards improvement, no marker was statistically significant. There was improvement in other patient questionnaire forms including the Duke Activity Status Index metabolic equivalents (3.4 [2.9–4.2] versus 3.9 [3.3–4.6]; $p = 0.0002$), Patient Health Questionnaire-9 (6.2 [3–8.5] versus 4.5 [3.5–6.5]; $p = 0.02$), MCS (48.6 [44.3–53.7] versus 52.5 [48.5–57.4]; $p = 0.003$), and PCS (40.8 [31.9–47.9] versus 44.2 [40.7–51.9]; $p = 0.003$) (Table 1).

The results of Pearson's correlation analysis for Duke Activity Status Index are summarised in Table 2. Percent of predicted peak oxygen consumption ($r = 0.49$, $p < 0.0001$), 6-minute walk distance ($r = 0.45$, $p < 0.0001$), 6-minute walk metabolic equivalents ($r = 0.45$, $p < 0.0001$), sit to stand repetitions ($r = 0.48$, $p < 0.0001$), dominant hand grip ($r = 0.48$, $p < 0.0001$), and physical component score ($r = 0.56$, $p < 0.0001$) were all associated with Duke Activity Status Index (Figure 2). On multivariable analysis, the percent of predicted peak oxygen consumption and dominant hand grip strength remained associated with the Duke Activity Status Index (Table 2).

Duke Activity Status Index was strongly associated with the Duke Activity Status Index metabolic equivalents ($r = 0.86$, $p < 0.0001$), and the Duke Activity Status Index metabolic equivalents were associated with the 6-minute walk metabolic equivalents ($r = 0.48$, $p < 0.0001$).

There were no associations between the change in Duke Activity Status Index (absolute and percent of predicted) and the objective measures of fitness obtained in this study.

When focused only on patients with CHD ($n = 62$), the median Duke Activity Status Index was 32.5 [24.2–50.7], and the average

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Table 1. Represented are the baseline demographics and outcomes for the entire cohort. Additionally, the demographics, baseline and final functional outcomes for the subset who completed the full cardiac rehabilitation program are included

	Total Cohort	Pre-CR	Post-CR	*p*-value
Total number	$n = 118$		$n = 33$ (CPET $n = 26$)	–
Age at start (years)	20 [13.9–22.5]		19.6 [14.1–21.4]	–
Less than 21 years	81 (69%)		24 (73%)	–
Sex (M/F)	63M/55F		19M/14F	–
Weeks completed (n)	13.2 [6.1–13.3]		20.9 [18.1–22.4]	–
Sessions completed (n)	24.2 [11–36]		35.0 [34–36]	–
Diagnosis	2V CHD – 42 1V CHD – 21 CM/HF – 53 PH – 2 EP – 1		2V CHD – 11 1V CHD – 6 CM/HF – 14 PH – 1 EP – 1	–
	Total Cohort	**Pre-CR**	**Post-CR**	***P*-value**
6 MW distance (m)	390.6 [308.5–470]	405.4 [310–490]	488.7 [383.5–563]	<0.0001
6 MW (METS)	2.9 [2.5–3.2]	2.9 [2.5–3.3]	3.3 [2.8–3.7]	<0.0001
Sit to stand (reps)	15.2 [11.3–19]	15.5 [12–19.5]	21.8 [17–27.5]	<0.0001
Arm curls (reps)	18.6 [16–21.5]	20.1 [17–23.8]	25.1 [19.3–30]	<0.0001
Sit and reach (cm)	39.4 [30.5–43.2]	38.1 [29.2–43.2]	42.9 [33.8–50.8]	0.005
Dominant HG (kg)	11.6 [7.2–15.4]	11.2 [7.0–16.1]	12.9 [7.3–17.8]	0.006
Indexed peak VO_2 (ml/min/kg)	20.8 [16–24]	21.9 [16–28.3]	22.6 [18–27]	0.9
% peak VO_2	60.1 [49–72.8]	63.1 [53–72]	69.9 [55–73]	0.4
Peak HR (bpm)	159 [142–181]	158.5 [137–184]	155.1 [134.5–178.3]	0.7
Duke	32.6 [21.5–48.5]	33.2 [24.2–46.7]	41.9 [31.5–53]	0.0005
Duke (% of total)	53.6 [40.0–85.9]	50.9 [45.0–84.1]	78.1 [54.3–100]	0.0002
Duke (METS)	3.4 [2.7–4.5]	3.4 [2.9–4.2]	3.9 [3.3–4.6]	0.0002
PHQ-9	5.5 [2–8.3]	6.2 [3–8.5]	4.5 [3.5–6.5]	0.02
MCS	48.5 [43.4–56.2]	48.6 [44.3–53.7]	52.5 [48.5–57.4]	0.003
PCS	40.3 [32.9–47.5]	40.8 [31.9–47.9]	44.2 [40.7–51.9]	0.003

Data are presented as a median[IQR] or absolute number (%). For comparison of the baseline and final cardiac rehabilitation outcomes, a paired *t* test was performed to compare before and after cardiac rehabilitation outcomes. $P < 0.05$ was considered significant.
CPET = cardiopulmonary exercise test; M = male; *F* = female; 2V = 2 ventricle; CHD = congenital heart disease; 1V= single ventricle, CM = cardiomyopathy; HF = heart failure; EP = electrophysiology; PH = pulmonary hypertension; SS = subglottic stenosis; 6MW = 6-minute walk; m = meters; reps=repetitions; cm = centimeters; HG = hand grip; VO_2 = oxygen consumption; ml= milliliters; min = minute; kg = kilogram; HR = heart rate; bpm = beats per minute; Duke = Duke Activity Status Index; METS = metabolic equivalents; PHQ-9=Patient Health Questionnaire-9; MCS = mental component scoring; PCS = physical component scoring.

median Duke Activity Status Index percent of total was 61.3 [41.6–87.1] %. Median peak oxygen consumption was 64.0 [52.8–74.0] % of predicted, or 18.7 [15.0–24.8] ml/kg/min. The Duke Activity Status Index remained associated with percent of predicted peak oxygen consumption ($r = 0.52$, $p = 0.0003$), 6-minute walk distance ($r = 0.49$, $p < 0.0001$), sit to stand repetitions ($r = 0.49$, $p < 0.0001$), dominant hand grip ($r = 0.49$, $p < 0.0001$), and physical component score ($r = 0.53$, $p < 0.0001$).

Discussion

This study compared perceived measures of fitness (using the Duke Activity Status Index) to actual measures of fitness in a population of youth and young adults with cardiac disease including many quantitative measures collected during cardiac rehabilitation. The Duke Activity Status Index was associated with 6-minute walk distance, the 6-minute walk test metabolic equivalent changes, and the Duke Activity Status Index metabolic equivalents in youth and young adults with heart disease. Additionally, the Duke Activity Status Index was also independently associated with peak oxygen consumption and maximal dominant hand grip. Lastly, though this cohort had a low cardiac rehabilitation completion rate, multiple measures of actual and perceived fitness increased in those who completed at least 12 weeks of cardiac rehabilitation. That resulted in a 31% increase in the number of patients who scored over 34 on the Duke Activity Status Index with this value having prognostic significance in adults with heart disease.[15]

Cardiac rehabilitation improves objective measures of fitness.[8,9,19,20] In addition to again demonstrating such objective benefit, this study shows that cardiac rehabilitation also improves subjective perceptions of fitness assessed by the increase in the Duke Activity Status Index. Patients with CHD often have poor perceptions of their fitness.[6,21] Improving perceptions of fitness is

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Table 2. Results of both the univariable and multivariable analyses for Duke Activity Status Index percent of total

Univariable Analysis	*r*	*p* value
Age	0.11	0.1
Height	0.37	<0.0001
Weight	0.42	<0.0001
BMI	0.39	0.0004
Peak VO_2 (%)	0.49	<0.0001
Peak HR	0.35	0.005
6 MW distance	0.45	<0.0001
6 MW (METS)	0.45	<0.0001
Sit and reach distance	−0.041	0.7
Sit to stand	0.48	<0.0001
Arm curls	0.43	<0.0001
Dominant HG	0.48	<0.0001
MCS	−0.0034	0.9
PCS	0.56	<0.0001
PHQ-9	−0.033	0.9
Multivariable analysis	**Standard-b coefficient (parameter estimates)**	***p* value**
Peak VO_2 %	0.40 (0.62 ± 0.21)	0.0047
6 MW distance	−0.056 (-0.017 ± 0.77)	0.7
Arm curls	0.030 (0.20 ± 0.88)	0.8
Sit to stand	−0.0058 (-0.033 ± 0.77)	0.96
Dominant HG	0.37 (0.86 ± 0.30)	0.0054

Univariable analysis was performed with Pearson's correlation coefficient. Candidate predictors for multivariable modeling included peak oxygen consumption, 6-minute walk distance, arm curls, sit to stand, and dominant handgrip. p value<0.05 was considered significant.
BMI = body mass index; VO_2=oxygen consumption; HR = heart rate; 6MW = 6-minute walk; METS = metabolic equivalents; HG = hand grip; MCS = mental component scoring; PCS = physical component scoring; PHQ-9=Patient Health Questionnaire-9.

important as youth with positive mindsets tend to have healthier exercise habits and higher levels of fitness have been associated with reduced morbidity and mortality in CHD.[22–26] Our study showed that the Duke Activity Status Index was associated with multiple measures of fitness and that through exercise therapy both actual and perceived fitness can improve. This differs from previous research that has shown no correlation with subjective measures of fitness and exercise capacity.[27] Potential explanations for the differences in findings between our cohort and the study from Burns R. et al (2010) include a lower power in our cohort, different subjective questionnaires for each study, and our cohort being an entirely cardiac rehabilitation cohort that generally had quite poor fitness. Additionally, the improvement of the Duke Activity Status Index above previously shown cut-offs may infer prognostic protections; however, this should be confirmed in larger studies.[15] On the other hand, the improvement in in Duke Activity Status Index may be secondary to either neuromuscular adaptation to exercise or could be a reflection of patient motivation to perform, which may be supported by the lack of improvement in the peak oxygen consumption following cardiac rehabilitation.

As perceptions correlate to actual fitness, mental training should be prioritised as much as physical training for CHD patients in cardiac rehabilitation.[7] The patients who have a healthier sense of self are better prepared to have improvements in physical activity, quality of life, and overall physical and mental health. Unfortunately, children and adults with CHD are at increased risk for many different forms of mental health difficulties including anxiety and depression.[28–30] This highlights the importance of regular mental health screening and interventions in this population. Cardiac rehabilitation may have a unique role to play in addressing some of these mental health concerns, provided that mental health screening and mental fitness training are integrated into the programme.[31,32] This is supported in our cohort by the significant improvements in the Patient Health Questionnaire-9 screen and the Mental Component Score following cardiac rehabilitation. These improvements could possibly be even greater should there be further integration of psychological services with the cardiac rehabilitation program, and this should be evaluated in future studies.

The Duke Activity Status Index is validated in adult heart failure patients.[2–4,16] This study shows that the questionnaire can also be informative for children and young adults with heart disease. It correlates with 6-minute walk distance and 6-minute walk metabolic equivalent just like in other non-CHD populations.[16,33,34] It also correlates with indexed peak oxygen consumption similar to other studies that have shown an association with peak oxygen consumption.[4,16,33] There were associations with the Duke Activity Status Index and maximum handgrip seen in our cohort, providing even more evidence that this fitness perception questionnaire correlates with multiple other aspects of actual fitness. This survey has been advocated to be used to risk-stratify adult patients before cardiac surgery.[16] As this survey is quick and easy to administer, there may be a role in administering the Duke Activity Status Index as a screener for low fitness in heart disease clinics. Should a patient score abnormally low, that could then in turn trigger further investigations (such as a cardiopulmonary exercise test) and possibly even referrals to cardiac rehabilitation.

There were several limitations to this study other than the inherent limitations of a single-site retrospective cohort. This is a selected sample in which there was a minority of patients who completed a full cardiac rehabilitation program and also underwent cardiopulmonary exercise testing. Those who complete cardiac rehabilitation and return for exercise testing are likely different from those who do not, thus introducing potential sampling bias. Another limitation includes no assessment of activity level outside of cardiac rehabilitation, as no exercise prescriptions or accelerometers were given in this study, so patients may have had different levels of exercise stimuli. Future studies could assess whether the Duke Activity Index Status is associated with future morbidity and mortality in this population of children and young adults with heart disease. Lastly, the Duke Activity Status Index is a validated instrument in adults but has not been validated in paediatric populations nor has it been validated when the question regarding sexual performance is removed. This should be researched in future studies.

In conclusion, cardiac rehabilitation improves perceptions of fitness as well as objective measures of fitness in children and young adults with heart disease. The Duke Activity Status Index is associated with multiple functional outcomes including 6-minute walk distance, hand grip, and peak oxygen consumption, and may help assess perceptions of fitness in those youth and young adults with heart disease.

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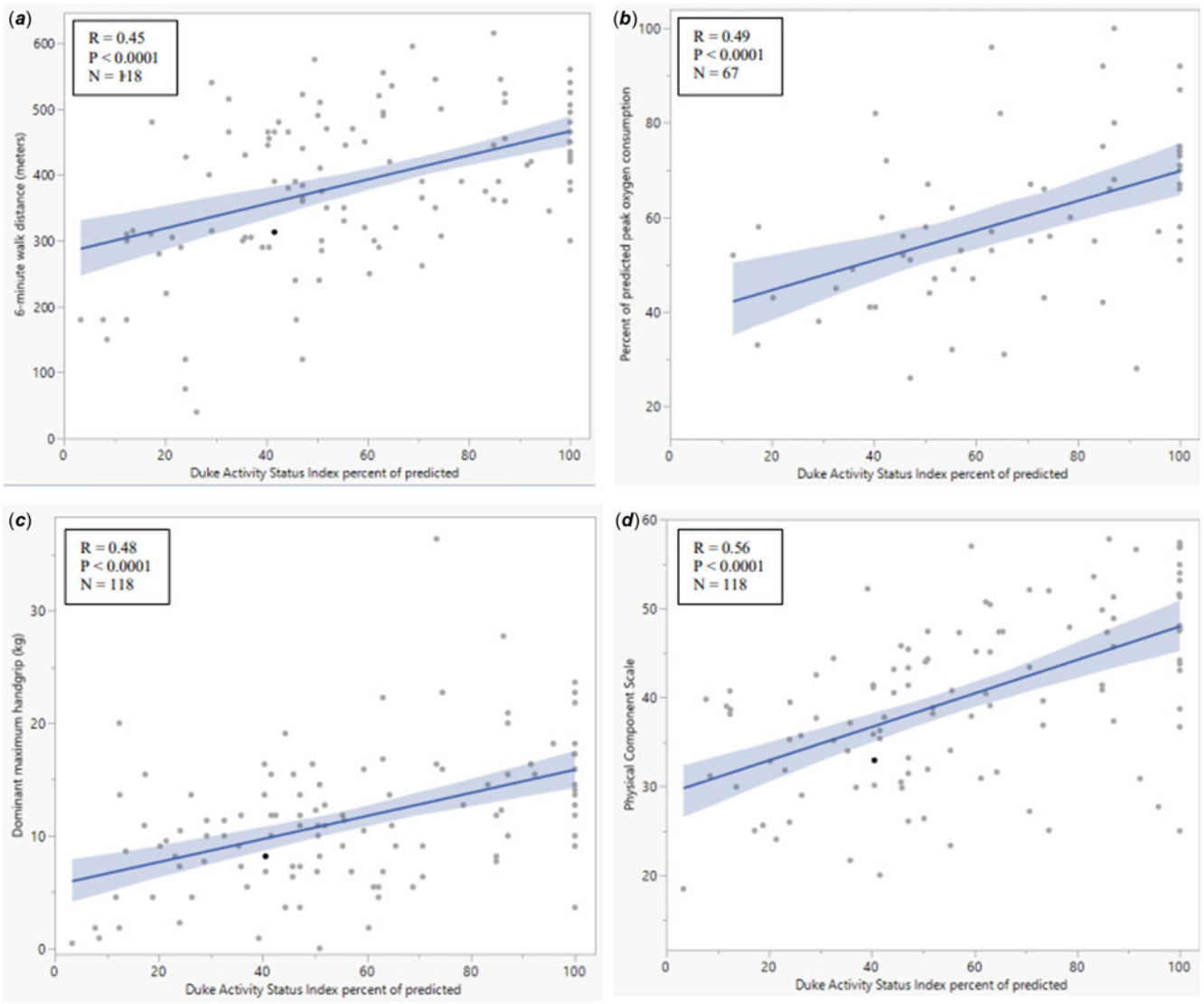

Figure 2. Correlations with line of best fit for Duke Activity Status Index percent of predicted normal and 6-minute walk distance (**2*A***), percent of predicted peak oxygen consumption (**2*B***), dominant hand grip (**2*C***), and PCS (**2*D***). Univariable analysis was performed with Pearson's correlation coefficient. *p*-value<0.05 was considered significant.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1047951124025939.

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Competing interests. The authors have no relevant financial or non-financial interest to disclose.

Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by institutional committees (Institutional Review Board at Cincinnati Children's Hospital Medical Center).

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