European Journal of Neurology

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE OPEN ACCESS

Preventing Cardiomyopathy in Duchenne Muscular Dystrophy: Long-Term Follow-Up of Patients in the Randomised, Placebo-Controlled Drug-Trial of Perindopril and Bisoprolol

John P. Bourke[1,2] | Andrew Bryant[3] | Gregory Landon[4] | Alexis Burn[5] | Stefan Spinty[6] | Ros Quinlivan[7] | Zoya Alhaswani[8] | Thomas Chadwick[3] | Francesco Muntoni[4] | Michela Guglieri[2] | DMD *Heart Study* Group

[1]Department of Cardiology, Freeman Hospital, Newcastle Hospitals Foundation Trust & John Walton Muscular Dystrophy Research Centre, Newcastle University, and Newcastle Hospitals Foundation Trust, Newcastle upon Tyne, UK | [2]John Walton Muscular Dystrophy Research Centre, Newcastle University, and Newcastle Hospitals Foundation Trust, Newcastle upon Tyne, UK | [3]Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, UK | [4]NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, London, UK | [5]Newcastle Joint Research Office, Newcastle University/The Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK | [6]Department of Paediatric Neurology, Alder Hey Children's NHS Foundation Trust, Liverpool, UK | [7]Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery. Queen's Square, London, UK | [8]Department of Paediatrics & Child Health, Birmingham Heartlands Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

Correspondence: John P. Bourke (john.bourke@nhs.net)

Received: 8 November 2024 | **Revised:** 6 February 2025 | **Accepted:** 17 February 2025

Funding: This study was funded by Duchenne UK (charity number: 1147094)—grant: 2021-06 NU-006994. DUK had no role in the design, data collection, data analysis, or reporting of the study.

Keywords: ACE-inhibitor | beta-blocker | cardiomyopathy prevention | DMD | heart medications | prophylaxis

ABSTRACT

Introduction: It is uncertain whether using cardiac drugs prophylactically in combinations for DMD is better than ACE-inhibitor alone. Our previous study showed no differences in left ventricular function between perindopril-bisoprolol and matched placebo after 36 months.

Methods: This study aimed to determine whether heart measures diverged after 60-month total follow-up. All participants had commenced open-label perindopril and bisoprolol when the original study ended. All were reconsented for access to heart measures, undertaken as part of their clinical care. The primary outcome was the change in echo-measured ventricular ejection fraction from baseline according to original randomization.

Results: Of 75 participants reported originally, 65 (aged 16 ± 2.5 years) were re-recruited and had data for analysis. Adjusted primary outcomes included 44 participants (original arms: 'active' 21; 'placebo' 23), 48 for secondary outcomes, and 65 for 'head-count' analysis of those with ventricular dysfunction. Absolute LVEF% values reduced in both groups ('active': 62.5% ± 5.6% to 53.8% ± 4.0%; 'placebo': 60.6% ± 4.9% to 50.4% ± 8.5%). Despite trends favoring earlier introduction of therapy, change from baseline was similar between groups (adjusted mean difference: -7.7 (95% CI -16.4 to1.0%)). However, more in the 'placebo' arm had died, had reduced LVEF%, and were taking additional heart medications.

Francesco Muntoni and Michela Guglieri are joint senior authors and contributed equally to the manuscript.

Andrew Bryant, Thomas Chadwick, Undertook and responsible for all statistical analyses.

Conclusion: While some patients may have benefited from 'early' (active) as opposed to 'delayed' (placebo) initiation of perindopril and bisoprolol, group-mean ventricular function did not differ between study arms after 60 months. Small numbers, absence of a control group, insensitivity of echo-ejection fraction, and additional drug use probably prevented divergence between groups.

1 | Introduction

Duchenne muscular dystrophy (DMD) is an X-linked, genetically inherited muscle wasting disorder and variously has other multi-system manifestations [1]. The clinical phenotype is caused by the absence of the protein dystrophin on muscle cell membranes, which makes myocytes uniquely vulnerable to damage with chronic secondary inflammation. This overcomes the capacity of repair mechanisms, leading to progressive loss of myocytes and fibro-fatty replacement of muscle tissues [2, 3]. The clinical phenotype of DMD is characterised by progressive skeletal, respiratory, and cardiac muscle weakness, with median life expectancy in the UK only 28.1 years, despite best multi-disciplinary care [4–6]. A dilated form of cardiomyopathy (DCM) is an inevitable complication of DMD, but unless it is sought, it remains asymptomatic until almost all heart function has been lost and heart failure symptoms emerge [7–9]. Although cardiac myocytes are abnormal from an early stage of DMD, the age at which left ventricular (LV) dysfunction becomes apparent on echo imaging is highly variable and occurs significantly later in those receiving corticosteroid therapy [5, 6, 9–12]. When cardiac magnetic resonance imaging (cMRI) is used in heart surveillance, myocardial fibrosis can be seen in a typical posterolateral basal, epicardial distribution even before LV-systolic dysfunction is detectable [13, 14].

Based mainly on the findings of one randomised placebo-controlled trial [15] but supported by various retrospective cohort series and meta-analyses [9, 16–20], DMD Care Standards recommend starting angiotensin-converting enzyme inhibitor (ACEi) medication empirically no later than the age of 10 years [7]. However, because evidence for routine use of an ACEi in young boys with DMD prophylactically was considered weak, in 2011 our group embarked on the double-blind, randomised, placebo-controlled *DMD Heart Protection* trial to test whether starting the combination of perindopril and bisoprolol (BB) in boys with DMD, aged 5–13 years, with normal LV-systolic function on echocardiographic (echo) assessment delayed the onset of LV-dysfunction or slowed its rate of progression when compared to placebo [21]. After 36 months, there was no group difference in the change in LV-ejection fraction (LVEF%) from baseline between active and placebo-treated participants [22]. However, at the end of the study, many participants had not reached the age at which LV-dysfunction is detectable on echo-assessment [5, 11]. The aim of this follow-up study was to determine whether differences in change from baseline LV-ejection fraction (LVEF%) could be detected between the groups after cumulative follow-up of 60 months.

2 | Methods

This was a separately registered [ISRCTN: 43827539], retrospective data collection study of heart outcomes in subjects who completed the previous, randomised, double-blind, placebo-controlled *Duchenne Muscular Dystrophy Heart-Protection Study* [EudraCT: 2007–005932-10; 2011-18], described and reported previously [20, 21]. In compliance with the study protocol and with recommendations of the *International Care Standards*, all patients were prescribed open-label ACEi and beta-blocker (BB) medication as they exited the original study, regardless of LV-function measurements. Patients with DMD are also recommended annual cardiac assessment as part of their multidisciplinary care [1, 7]. For this follow-up study, data were obtained retrospectively from the cardiac assessments patients had undergone as part of their routine care from the time they exited the original study (i.e., their last assessment visit). No additional testing or hospital visits were required, and there was no restriction preventing cardiologists from adding other cardioactive medications, either prophylactically or for ventricular dysfunction, at their clinical discretion during the period from which results were obtained.

2.1 | Inclusion and Exclusion Criteria

To be included in the study, patients had to have completed 3 years in the original *DMD Heart-Protection Study* or have already reached a study endpoint within that time. Patients/parents or guardians, depending on age, had to formally consent to allow researchers access retrospectively to the results of all heart assessments and limited other data, amassed in the period March 2018 to November 2023, from clinical records at paediatric or adult cardiology clinics at specialist or district hospital sites. Patients were excluded if they had not completed 3 years in the original *DMD Heart Protection Study*, if they could not be contacted by the research team, or if they declined consent.

2.2 | Primary and Secondary Outcome Measures

The primary study endpoint was change in LVEF% from original study enrolment ('baseline') after an average of 60 months of follow-up, according to initial treatment randomisation (active vs. placebo). Secondary endpoints were change in LV-fractional shortening (LVFS%), LV end-diastolic (LVEDd) and end-systolic (LVESd) dimensions and/or volumes and tissue-Doppler measures.

Since there was no longer a placebo group in this follow-up study, the primary comparison was to determine whether 'early' (prophylactic; referred to as 'active') introduction of the perindopril-bisoprolol combination preserved LVEF% better than 'delayed' initiation (referred to as 'placebo') of the same medications some 3 to 5 years later. The *null hypothesis* was that the ACEi-BB combination would not slow the rate of decline in

LV function between those randomised to 'early' as compared to 'delayed' initiation of the same therapy over a total follow-up of 60 months.

2.3 | Measures of Exposure

A document of the variables to be sought retrospectively from each cardiac assessment was agreed upon by principal investigators (PIs) in advance. Echo-measures and limited other data was collected by researchers reviewing patients case records on each of the four study sites. In the case of those whose cardiac care had transferred to another hospital (eg: paediatric to adult clinic), researchers from the study site requested more recent results from where tests had been performed.

2.4 | Study Population, Obtaining Consent and Recruitment

Original contact details for all participants in the original study were known to research teams on each of the four participating UK NHS hospital sites. This information was used to reestablish contact with patients/families. The study was explained with the aid of a *Patient Information Sheet*, and patients/parents or guardians were reconsented to allow researchers access to clinical records and to extract results of all heart tests performed as part of their routine clinical care. Ethics approval allowed inclusion of data on those who had died, without seeking reconsent from families.

2.5 | Statistical Analysis

A statistical analysis plan was formulated ahead of locking the data for analysis. Delivery of care restrictions during the COVID pandemic prevented heart assessments altogether in the period 2020-'21 and, thereafter, assessments occurred at varying intervals rather than strictly annually. Therefore, to maximize the amount of data available for inclusion in the final analysis, group measures of heart function were compared at only one time point, using a 60–72-month follow-up 'window' (i.e., '60 months'). Descriptive summary statistics are presented for baseline characteristics and longitudinal outcomes up to a minimum 60 months as reported. For the outcome comparisons after a mean 60-month follow-up, the heart assessment nearest to that time point (± 12 months) was used. For the primary outcome, differences at 60–72 months from the original baseline between 'active' ('early' therapy initiation) and 'placebo' ('delayed' therapy initiation) groups were estimated using analysis of covariance (ANCOVA). ANCOVA was repeated to estimate differences between treatment groups for secondary endpoints of LV fractional shortening, LV dimensions, and tissue Doppler measures of segmental LV function. When data allowed, pre-specified sub-group comparisons of LVEF% were performed: (i) steroid-using versus steroid-naive patients; (ii) change in LVEF% by age at the time of end-analysis (iii) 'headcount' comparison of patient numbers at various severities of LV dysfunction; (iv) lowest LVEF% at any time point and (v) use of additional heart medications by the time of end analysis. All analyses used groups defined by intentiontotreat.

2.6 | Sample Size

The sample size was dictated by the original number recruited [$n = 85$], who had completed the three-year follow-up [$n = 74$] and the number who could be recontacted and consented to participate.

2.7 | Ethical Approval

The study received ethical approval from *London—Brent Research Authority* [ID 269110; 09825], was funded by *Duchenne UK*, a registered DMD charity in the United Kingdom, and Newcastle upon Tyne Hospitals NHS Foundation Trust was the study sponsor.

2.8 | Data Handling

Participants were identified only by their original study enrolment identifiers to ensure data security/confidentiality. All data was pseudo-anonymised and uploaded to a dedicated, secure, online research portal (*RedCap*: Research Electronic Data Capture, Vanderbilt University, USA), making it available to the research team centrally for analysis. Data will be stored and archived securely according to good clinical practice.

3 | Results

Sixty-five of the 74 participants who completed the 36-month follow-up of the *DMD Heart Protection Study* were recontacted and consented to participate in this longer-term follow-up study. The reduced number is explained by the inability to reestablish contact with families ($n = 5$), by those who had either died ($n = 1$) or who had already reached a study endpoint ($n = 4$) during the original study (Figure 1) [22]. Eight more patients died before reaching the minimum '60-month' follow-up—two randomized originally to active and seven to placebo therapy. Three deaths in the 'placebo'- but none in the 'active'-treatment group were either definitely ($n = 1$) or probably ($n = 2$) cardiac in nature. Their mean LVEF% was 37% as compared to 60% in those who died from non-cardiac causes.

Analyses at 60 months included comparisons for changes in LVEF% - the primary endpoint, in 44 participants (original active arm 21; placebo arm 23) and for up to 48 participants for other secondary outcome measures (i.e., LVFS%; LV-chamber dimensions). Data were available for all 65 patients (including 20 participants from the primary phase of the trial) to compare the number of participants in each study arm with reduced LVEF% at various thresholds of abnormality and for 78 originally recruited participants for a 'lowest LVEF% at any timepoint' comparison.

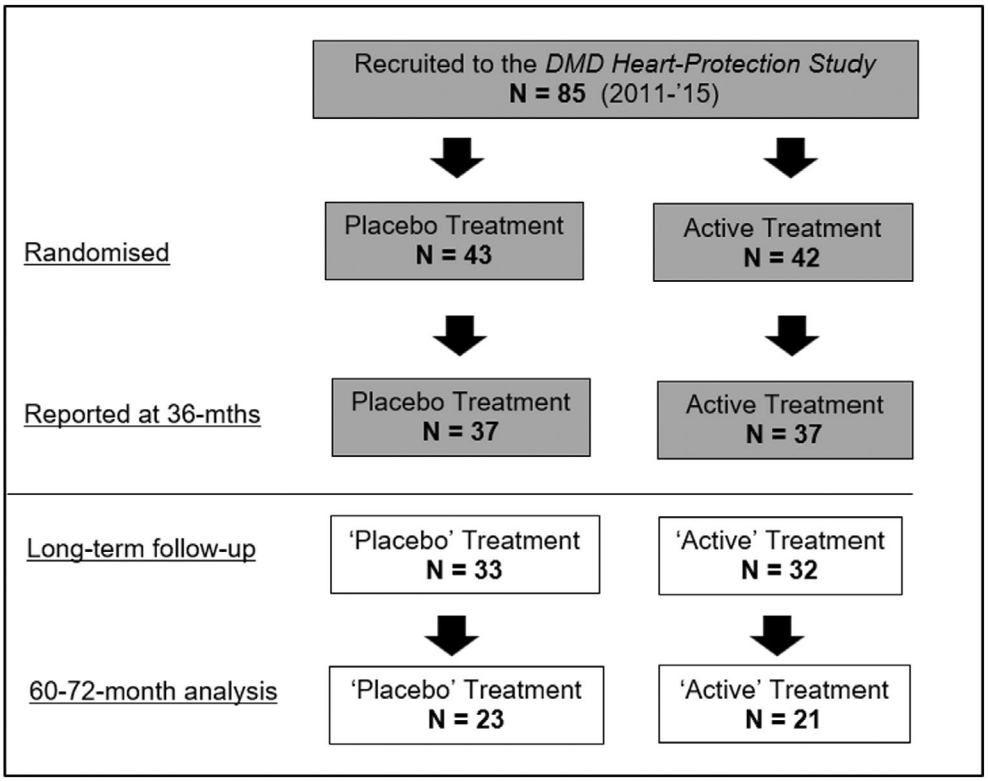

FIGURE 1 | Participant flow through original and follow-up phases of the study.

TABLE 1 | Participant age distribution at initial randomisation and at time of final analysis.

	'Active' ('early') treatment		'Placebo' ('delayed') treatment	
Categorical variables	*n*	*n* = 32	*n*	*n* = 33
Age (years) at randomisation				
5	0	—	0	—
6	0	—	1	3.03%
7	8	25%	6	18.18%
8	6	18.75%	6	18.18%
9	7	21.88%	5	15.15%
10	6	18.75%	7	21.21%
11	2	6.25%	2	6.96%
12	3	9.38%	3	9.09%
13	0	—	3	9.09%

Continuous variables	***n***	**Mean ± SD**	**Median (IQR)**	**Range**	***n***	**Mean ± SD**	**Median (IQR)**	**Range**
Age (years) at randomisation	32	**9.4 ± 1.7**	9.5 (8.0–10.6)	7.0–12.5	33	**9.8 ± 2.1**	9.7 (8.4–10.7)	6.1–13.8
Age (years) at 60–72-month analysis	32	**15.5 ± 2.0**	15.3 (13.9–16.6)	12.8–20.3	33	**16.4 ± 2.9**	15.8 (14.3–18.7)	12.0–22.5

Abbreviations: 'Active' ('early') treatment = those randomised to perindopril/bisoprolol initially, *N* = number of participants, 'Placebo' ('delayed') treatment = those randomised to placebo initially and taking open labelled perindopril/bisoprolol from end of original study, QR = inter-quartile range, SD = standard deviation.

Table 1 summarises the age distribution of participants at original randomisation and after 60-month follow-up. Group mean ages at the point of final analysis in 'active' ('early' therapy) and 'placebo' ('delayed' therapy) groups were not significantly different (15.5 ± 2.0 and 16.4 ± 2.9 years respectively). Table 2 shows the spread in follow-up durations at the time of analysis, and Table 3 lists all cardioactive medications being taken by patients at the time of last assessment.

TABLE 2 | Durations of participant follow-up with cardiac data for inclusion in final analysis.

	'Active' ('early') treatment		'Placebo' ('delayed') treatment		Total	
Follow-up (years)	***N***	**%**	***N***	**%**	***N***	**%**
5	5	15.62	3	9.09	8	12.31
5.5	6	18.75	7	21.21	13	20
6	10	31.25	13	39.39	23	35.38
6.5	3	9.38	3	9.09	6	9.23
7	5	15.62	0	0	5	7.69
7.5	2	6.25	2	6.06	4	6.15
8	0	0	1	3.03	1	1.54
9.5	0	0	1	3.03	1	1.54
10.5	0	0	2	6.06	2	3.08
11	1	3.12	0	0	1	1.54
12	0	0	1	3.03	1	1.54
Total	**32**		**33**		**65**	

Abbreviations: 'Active' ('early') treatment = those randomised to perindopril/bisoprolol initially, *N* = number of participants, 'Placebo' ('delayed') treatment = those randomised to placebo initially and taking open labelled perindopril/bisoprolol from end of original study.

3.1 | Heart Rates and Blood Pressures as Indicators of the Adequacy of Therapy Dosing

Consistent with differences in therapy randomisation during the first 36 months of the original study, heart rates (HR) and systolic and diastolic blood pressures (BP) were lower in those randomised to active as compared to placebo therapy (HR active: 84.4 ± 16.7 versus placebo: 95.8 ± 13.2 beats/min and systolic BP active: 104.6 ± 12.0 versus placebo: 114.4 ± 11.0 mmHg; diastolic BP active: 62.3 ± 10.1 versus placebo: 69.3 ± 10.0 mmHg). During the follow-up phase, the lowest recorded mean heart rates and systolic blood pressure were not significantly different between the groups (HR active: 75 ± 11 versus placebo: 78 ± 17 beats/min and systolic BP active: 106 ± 13 versus placebo: 114 ± 10 mmHg). Diastolic blood pressure was higher in the placebo group (active: 62 ± 8 versus placebo: 70 ± 9 mmHg; $p = 0.006$).

3.2 | Change in LVEF% From Original Randomisation

Group mean LVEF% had decreased in both study arms, but the change from recruitment to final analysis at '60-months' was not significantly different between active ('early') and placebo ('delayed') treatment groups (Table 4).

3.3 | Comparison in Secondary Cardiac Outcome Measures

No significant differences were found either in any of the secondary endpoints: LVFS%, LV-end diastolic (LVEDD) or LV-end systolic dimensions (LVESD) between active ('early') and placebo ('delayed') treatment groups (Table 5). Tissue-Doppler measures were only available from a limited number of assessments, so a comparison of posterolateral/postero-basal LV-segmental dysfunction between groups was not possible (i.e., lateral to septal LV-wall S′ measurements).

3.4 | Other 'Headcount' Comparisons of LVEF% Between Groups

On the basis that starting active treatment in those previously randomised to placebo might correct a greater prior deterioration in LV function, an additional analysis compared groups according to each patient's lowest recorded LVEF% at any timepoint. Table 6 shows 'headcount' comparisons of participants included in the '60-month' analysis whose LVEF% had dropped below various abnormal thresholds. There were consistently more in the placebo group at all levels of abnormality. Although there was a trend in favour of the group starting therapy earlier (i.e., 'active' group) having better preserved LVEF%, the difference was not clinically significant ['Active': $n = 40$; LVEF 47.9% ± 10.4%: 'Placebo': $n = 38$; LVEF 45.5% ± 9.9%; $t = 1.04$; $p = 0.15$].

3.5 | Pre-Specified Subgroup Analyses

3.5.1 | Corticosteroid Status

Of 44 participants with LVEF% measures at 60–72months, only nine (21%) were corticosteroid naïve, 34 (79%) were on long-term steroid therapy, and the corticosteroid status was uncertain in one. No difference in final LVEF% according to corticosteroid status was found (LVEF% steroid takers: 55.4% ± 8.1% versus steroid naïve 54% ± 13.3%; p = NS).

3.5.2 | Age

Of 44 patients with LVEF% measures at 60–72months, 26 (59%) were older and 18 (41%) younger than 15 years of age, and four were aged less than 13 years. Although both the difference in final LVEF% and change of LVEF% from baseline were greater between 'active' and 'placebo' groups in those aged 15 years and above, neither difference appeared clinically meaningful [(age ≥ 15 years: 52.9% ± 10.4%; age < 15 years: 57.5% ± 7%) and (change in LVEF% ≥ 15 years: −7.5% ± −11.8% and < 15 years: −3.8% ± −8.1%)].

3.5.3 | Addition of Other Cardioactive Medications

During the study, all patients were taking an ACE-inhibitor and beta-blocker drug combination, and some had a mineralocorticoid antagonist (MRA) added at the discretion of clinical teams (Table 3). More of those in the 'placebo' group had eplerenone or spironolactone added to their cardiac regimen, and in one other case, sacubitril-valsartan had been substituted for an ACEi prior to the 60-month assessment ['Active' 9 (28.1%): 'Placebo' 12 (36.4%)]. An MRA was being taken by more of those whose

TABLE 3 | Initial therapy randomization and heart medications at final analysis.

'Active' ('early') treatment	*n* (%)	'Placebo' ('delayed') treatment	*n* (%)
Phase 1[a]			
Perindopril and bisoprolol steroids[d]	42 34 (80.9%)	Matched placebo steroids[d]	43 31 (72.1%)
Phase 2[b]			
Perindopril and bisoprolol	32		33
Steroids[d]	28 (87.5%)		23 (69.7%)
Testosterone	15 (46.9%)		11 (33%)
Ataluren/exon-skipping[d]	2		0
Idebenone/givinostat[d]	1		1
Additional heart medications[c]			
Eplerenone/spironolactone	9 (28.1%)		11 (33%)
Dapagliflozin/furosemide	2		4
Sacubitril-valsartan	0		1 (3%)
Carvedilol, ivabradine, digoxin	1		2

Abbreviations: *N* = participant numbers, *p* = significance test value.
[a]Double-blind randomised phase, identical over-encapsulated active and placebo capsules.
[b]Open-labelled perindopril and bisoprolol.
[c]Not mutually exclusive.
[d]Taking maintenance glucocorticoid steroids for DMD.

TABLE 4 | Group comparison of primary outcome measure at 60–72 months (change in LVEF %).

	'Active' treatment			'Placebo' treatment			Difference up to long-term follow-up[c]
LVEF%	*n*	Mean[a] (SD)	Range	*n*	Mean[a] (SD)	Range	Adj. diff in means[b] (I-C) (95% CI)
Baseline (of those with longterm follow-up)	32	62.53 (5.62)	52-76	29	60.59 (4.87)	51-73	n/a
Long-term follow-up time point[d]	21	57.24 (7.21)	39-71	23	52.52 (10.61)	23-76	Adj. diff in means[b] −7.69 (−16.40 to 1.01)
Sensitivity analysis with: long-term followup time point[d,e]	21	53.81 (4.40)	39-56	23	50.43 (8.15)	23-56	Adj. diff in means[b] −5.64 (−13.56 to 2.26)

[a]Univariate analysis reporting change in mean and SD from baseline without adjustment.
[b]Multivariate analysis reporting difference in means between groups at long-term follow-up timepoint from baseline with adjustment for baseline value and baseline covariates including age (continuous), body surface area (continuous) and genetic mutation type (deletion vs. non-deletion). The unadjusted mean difference was −3.77 (−10.53 to 2.98).
[c]Multivariate analysis reporting the difference in means between groups at long-term follow-up timepoint from baseline with adjustment for baseline value and baseline covariates including age (continuous), body surface area (continuous) and genetic mutation type (deletion vs. non-deletion). The unadjusted mean difference was −2.19 (−7.85 to 3.48).
[d]Long-term follow-up ranged from 60 to 144 months (see Table 6 for breakdown).
[e]Using LVEF values that were capped at an upper range of 56 to account for any values classified as > 55%.

lowest LVEF was below 45% in the 'placebo' than in the 'active' treatment group ('Active' 3; 'Placebo' 5).

4 | Discussion

The main finding of this longer-term, follow-up phase of the *DMD Heart-Protection Study* is that, when started prophylactically in young boys (Active: 9.4 ± 1.7 & Placebo: 9.8 ± 2.1 years) with normal echo measures of LV function at baseline, the combination of perindopril and bisoprolol did not change the subsequent decline in group mean LVEF% over 60-month follow-up when compared to starting the same two medications 3 to 5 years later. The groups did not differ significantly either in any of the secondary end points—LVFS%, LVED- and LVES dimensions. There were insufficient LV tissue Doppler measurements for a valid analysis of segmental LV function.

Although the negative overall results appear to run counter to current *Standards of Care* recommendations, small numbers, no

TABLE 5 | Secondary cardiac outcome measures at 60-months.

Outcome (at 60-months)	Range	'Active' ('early') treatment		'Placebo' ('delayed') treatment		Difference
		n	Mean[a] (SD)	*n*	Mean[a] (SD)	Adj. diff in mean[b] (I-C) (95% CI)
LVESD (cm) Baseline	1.9-3.3	31	2.55 (0.3)	33	2.59 (0.3)	n/a
At 60-months[c]	1.7-5.43	24	2.98 (0.5)	23	2.90 (0.9)	**Adj. diff in means**[b] 0.18 (−0.38 to 0.75)
LVEDD (cm) Baseline	3-4.8	31	3.92 (0.4)	33	3.97 (0.3)	n/a
At 60-months[c]	2.7-6.09	24	4.28 (0.4)	24	4.23 (0.8)	**Adj. diff in means**[b] 0.02 (−0.51 to 0.56)
LVFS% Baseline	30-48	32	35.13 (3.4)	33	34.85 (4.3)	n/a
At 60-months[c]	11-68	20	36.5 (10.7)	21	34.65 (14.4)	**Adj. diff in means**[b] −4.11 (−12.83 to 4.61)

[a]Univariate analysis in mean and SD change at long-term follow-up time point from baseline without adjustment.
[b]Multivariate analysis reporting the difference in means between groups at long-term follow-up timepoint from baseline with adjustment for baseline value and baseline covariates including age (continuous), body surface area (continuous), and genetic mutation type. The unadjusted mean difference was −0.15 (−0.56 to 0.25), −0.17 (−0.52 to 0.18) and − 1.27 (−10.05 to 7.51) for LVESD, LVEDD, and LVFS, respectively.
[c]Long-term follow-up ranged from 60 to 144 months.

TABLE 6 | 'Headcount' of patients with abnormal LVEF% at '60-months' and group comparison of lowest recorded LVEF% at any timepoint.

LVEF% at '60-months'	'Active' ('early') treatment	'Placebo' ('delayed') treatment
< 55% (n=60)	28	32
< 50% (n=43)	20	23
< 45% (n=31)	15	16
Lowest LVEF% at any timepoint* (*n* = 78)	47.9% ± 10.4%	45.5% ± 9.9%

Abbreviations: '60-month' = patients with heart measures in the 60-72-month data-window, 'Active' ('early' therapy) = those randomised to perindopril and bisoprolol originally, LVEF% = left ventricular ejection fraction, *N* = participant number, 'Placebo' ('delayed' therapy) = those taking placebo during original study and open-labelled perindopril and bisoprolol during follow-up study.

longer having a placebo group, and the introduction of additional cardioactive medications will have contributed to minimizing differences between the groups. Interestingly, however, the findings are consistent with both previous placebo-controlled studies of heart medications for DMD, which were also small studies and not powered for mortality [7, 15, 23]. Those same factors explain why neither the French perindopril-versus-placebo study [15], in steroid-naïve patients, nor the German study [23], comparing the combination of enalapril and metoprolol with placebo in which 68% were also taking steroids, demonstrated group differences in primary study endpoints either [15, 23]. However, both showed beneficial trends for some participants who took active therapy either at a younger age or for longer. Evidence for similar trends is apparent also from subgroup analyses of this study's data, in which 77% of participants were also receiving corticosteroids for DMD. Although differences in the various analyses of LV-function (Table 6) are not statistically significant, group mean LVEF% was higher both at the 60-month mark and for the lowest recorded LVEF% at any time point during the study for those in the 'active' ('early') treatment group. More in the 'placebo' (delayed treatment) group had LVEFs below each of three thresholds—less than 55%, 50%, and 45%. Cardiac deaths occurred only in the placebo group, and more patients died from all causes in the 'placebo' ('delayed') treatment group. Similarly, in the French perindopril study, three participants, all with LVEF less than 45%, died [15]. These trends are concordant with the findings of this study and support the routine introduction of the perindopril-bisoprolol combination prophylactically for its long-term outcome benefits.

4.1 | Significance of Various Confounding Factors on the Overall Group Results

Some confounding factors would have been expected to prevent greater divergence in heart function between 'active' and 'placebo' treated participants. Because there was no longer a placebo-treated group, the benefits for the 'placebo' group of receiving open-labelled perindopril/bisoprolol probably reduced differences in endpoints, which might have emerged if a true placebo comparison had been ethically possible.

Most patients were receiving steroids for muscle strengthening in DMD and that also delays the onset of cardiomyopathy by an average of 2 years compared to those who are steroid naïve [5, 24–27]. Previously our group reported that the age of detection of LV-dysfunction by echo was 15.1 ± 4.2 years—but older in those on maintenance steroid therapy (16.8 + 4.2 versus 14.5

±4.1 years; $p = 0.04$) [11]. The age of participants at the '60-month' assessment was 15.5 ± 2.0 and 16.4 ± 2.9 years for 'active' and 'placebo' groups respectively, and 15 (33%) were still below 13 years of age. Although all participants would be expected to develop LV-dysfunction at some point, many still had either preserved or minimally reduced function at the '60-month' assessment. The implication is that they had yet to develop the degree of echo-detectable LV-dysfunction expected at some point of follow-up in all patients with DMD and which could allow divergence between the groups [5, 25]. Furthermore, since some in the 'placebo' arm were less than age 13 when they started open-label therapy, their rate of decline in LV-function would be expected to follow that of those randomised originally to active therapy according to the original study inclusion criteria [21, 22].

This was a small study and, if the scale of added benefit from heart medications in patients receiving steroids is small, a much larger study or even longer follow-up would have been needed to confirm differences in outcome. Indeed, the benefits for the heart of steroids and the perindopril-bisoprolol combination may be qualitatively different and so, only emerge in the form of endpoints such as symptomatic heart failure or mortality, much later in the course of DMD [26–31]. More participants in the 'placebo' arm received additional cardiac medications, mineralocorticoid antagonists and/or sacubitril-valsartan and ivabradine, which would be expected to have slowed their decline in heart function [20, 32–34]. Furthermore, the difference in time between those receiving 'early' (initial active group) and 'delayed' (initial placebo group) was only about 3 years, which could have resulted in more equivalent treatment benefits from cardioactive medications than intended.

4.2 | Study Limitations

The main limitations of the study were, firstly, its small size and secondly, the relative insensitivity of echo compared to cardiac magnetic resonance (CMR) imaging in detecting changes in myocardial tissue composition and subtle early changes in cardiac function and remodelling in adolescent patients with DMD [13, 14, 35–37]. However, when the study was first conceived, CMR imaging was not widely available in UK hospitals and some of the more sensitive measures available now had not been validated for use as study end points.

5 | Conclusions

The main conclusion of this longer-term follow-up study of the original DMD-heart protection cohort is that, when started prophylactically in young boys with normal LV function and who were also receiving steroids, the combination of perindopril & bisoprolol did not change the subsequent decline in LVEF% over 60 months of follow-up compared to starting the same medications 3 to 5 years later. However, various trends favoring the introduction of prophylactic therapy were evident in sub-group analyses, supporting current DMD standards of care recommendations to introduce heart medications prophylactically. The perindopril-bisoprolol combination was well tolerated throughout, with no withdrawals because of intolerance or adverse effects. The study also highlights a range of considerations and confounders relevant to the design and conduct of future cardiac studies in the complex milieux of DMD.

Author Contributions

John P. Bourke: conceptualization, funding acquisition, writing – original draft, writing – review and editing, supervision, investigation, data curation, project administration. **Andrew Bryant:** methodology, validation, writing – review and editing, formal analysis, data curation, writing – original draft, supervision. **Gregory Landon:** investigation, validation, writing – review and editing, data curation, project administration. **Alexis Burn:** investigation, writing – review and editing, project administration, supervision. **Stefan Spinty:** investigation, validation, writing – review and editing, supervision, data curation, project administration. **Ros Quinlivan:** data curation, supervision, writing – review and editing, investigation, project administration. **Zoya Alhaswani:** investigation, data curation, writing – review and editing, validation, project administration. **Thomas Chadwick:** methodology, validation, writing – review and editing, formal analysis, data curation. **Francesco Muntoni:** conceptualization, supervision, writing – review and editing, methodology, project administration. **Michela Guglieri:** conceptualization, methodology, writing – review and editing.

Acknowledgements

The authors thank Duchenne UK for funding the research and wish to particularly thank the boys and their families for participating in the study—without their willingness to take part, the study could not have happened. The views expressed are those of the author(s) and not necessarily those of the NHS or Duchenne UK. DMD Heart Protection research group: Natasha Aslam, Leslie Bremner, David Grisdale, Vitalija Jakovlevaite, Anna Johnson, Trevor Liddle, Konstantinos Savvatis, and Hannah Stevenson.

Disclosure

Study Approval Statement: This study protocol was reviewed and approved by the *London—Brent Research Authority* [ID 269110; 09825], was registered with ISRCTN (registration number: 43827539) and Newcastle upon Tyne NHS Foundation Trust was the study sponsor (Joint Research Office: trust.r&d@nuth.nhs.uk).

Consent

All patients/patient families were required to give informed consent to enter the study. This was provided either in written format or in a confirmatory email or text message following a phone discussion with a member of the research team. To avoid causing families unnecessary distress, ethics approval allowed access to the data of those who had died without reconsent. No support from any organisation other than Duchenne UK [grant: 2021-06] for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Conflicts of Interest

John P. Bourke organisational grant from Duchenne UK to conduct this study. DMC participation fees from *Sarepta Therapeutics*; Travel support from *Sarepta Therapeutics*; consulting and advisory board fees from Sarepta Therapeutics, *Pfizer*, and the *Esperare* Foundation. Andrew Bryant (statistician) no conflicts of interest to declare. Gregory Landon no conflicts of interest to declare. Alexis Burn (trial manager) no conflicts of interest to declare. Stefan Spinty no conflicts of interest to declare. Ros Quinlivan reports receiving lecturing honoraria from *PTC Bio*, *Sanofi-Genzyme*, *Sarepta Therapeutics*, *Santhera*, *Roche*, and *Biogen*; DMC participation fees from *Roche*, *TRiNDs*, *Astellas*, *PTC Bio*,

Santhera, and *Sarepta Therapeutics*. Zoya Alhaswani reports travel support grant from Roche (unrelated to submitted work). Thomas Chadwick (statistician) no conflicts of interest to declare. Francesco Muntoni reports grants from *Sarepta Therapeutics* and Muscular Dystrophy UK; consulting fees from Roche and PTC; Travel support from *Sarepta Therapeutics* and Roche; DMC participation payments from *Pfizer*. Michela Guglieri reports acting as chair for a study sponsored by *ReveraGen* (no financial benefits); research collaborations with *ReveraGen, Sarepta Therapeutics*, and *Edgewise*; acting as CI/PI for clinical trials sponsored by *Dyne, Pfizer, Italfarmaco, Edgewise, Roche, Santhera, ReveraGen*, and *Dynacure*; participating in advisory boards for *Pfizer, NS Pharma, Dyne* (consultancies through Newcastle University); and receiving speaker honoraria from *Sarepta, Italfarmaco, Novartis, Roche*, and *Dyne*.

Data Availability Statement

All data accruing from this study have either been included in the manuscript or supplied in accompanying appendices.

References

1. D. J. Birnkrant, K. Bushby, C. M. Bann, et al., "Diagnosis and Management of Duchenne Muscular Dystrophy, Part 1: Diagnosis, and Neuromuscular, Rehabilitation, Endocrine, and Gastrointestinal and Nutritional Management," *Lancet Neurology* 17, no. 3 (2018): 251–267, https://doi.org/10.1016/S1474-4422(18)30024-3.

2. P. Dowling, D. Swandulla, and K. Ohlendieck, "Cellular Pathogenesis of Duchenne Muscular Dystrophy: Progressive Myofibre Degeneration, Chronic Inflammation, Reactive Myofibrosis and Satellite Cell Dysfunction," *European Journal of Translational Myology* 33, no. 4 (2023): 11856, https://doi.org/10.4081/ejtm.2023.11856.

3. F. Gatto, S. Benemei, G. Piluso, and L. Bello, "The Complex Landscape of DMD Mutations: Moving Towards Personalized Medicine," *Frontiers in Genetics* 15 (2024): 1360224, https://doi.org/10.3389/fgene.2024.1360224.

4. Z. Koeks, C. L. Bladen, D. Salgado, et al., "Clinical Outcomes in Duchenne Muscular Dystrophy: A Study of 5345 Patients From the TREAT-NMD DMD Global Database," *Journal of Neuromuscular Diseases* 4, no. 4 (2017): 293–306, https://doi.org/10.3233/JND-170280.

5. J. Broomfield, K. Abrams, N. Latimer, M. Guglieri, M. Rutherford, and M. Crowther, "Natural History of Duchenne Muscular Dystrophy in the United Kingdom: A Descriptive Study Using the Clinical Practice Research Datalink," *Brain and Behavior: A Cognitive Neuroscience Perspective* 13, no. 12 (2023): e3331, https://doi.org/10.1002/brb3.3331.

6. F. J. Weber, T. D. Latshang, M. R. Blum, M. Kohler, and M. M. Wertli, "Prognostic Factors, Disease Course, and Treatment Efficacy in Duchenne Muscular Dystrophy: A Systematic Review and Meta-Analysis," *Muscle & Nerve* 66, no. 4 (2022): 462–470, https://doi.org/10.1002/mus.276.

7. D. J. Birnkrant, K. Bushby, C. M. Bann, et al., "Diagnosis and Management of Duchenne Muscular Dystrophy, Part 2: Respiratory, Cardiac, Bone Health, and Orthopaedic Management," *Lancet Neurology* 17, no. 4 (2018): 347–361, https://doi.org/10.1016/S1474-4422(18)30025-5.

8. A. Fayssoil, S. Abasse, and K. Silverston, "Cardiac Involvement Classification and Therapeutic Management in Patients With Duchenne Muscular Dystrophy," *Journal of Neuromuscular Diseases* 4, no. 1 (2017): 17–23, https://doi.org/10.3233/JND-160194.

9. F. Kamdar and D. J. Garry, "Dystrophin-Deficient Cardiomyopathy," *Journal of the American College of Cardiology* 67, no. 21 (2016): 2533–2546, https://doi.org/10.1016/j.jacc.2016.02.081.

10. J. P. Bourke, M. Guglieri, D. Duboc, and ENMC 238th Workshop Study Group, "238th ENMC International Workshop: Updating Management Recommendations of Cardiac Dystrophinopathy Hoofddorp, The Netherlands, 30 November - 2 December 2018," *Neuromuscular Disorders* 29, no. 8 (2019): 634–643, https://doi.org/10.1016/j.nmd.2019.06.598.

11. A. P. Murphy, A. Johnson, V. Straub, A. Heads-Baister, S. Lord, and J. P. Bourke, "Effects of Cardiac Medications on Ventricular Function in Patients With Duchenne Muscular Dystrophy-Related Cardiomyopathy," *Muscle & Nerve* 64, no. 2 (2021): 163–171, https://doi.org/10.1002/mus.27336.

12. M. Schiava, R. M. Lofra, J. P. Bourke, et al., "Functional Abilities, Respiratory and Cardiac Function in a Large Cohort of Adults With Duchenne Muscular Dystrophy Treated With Glucocorticoids," *European Journal of Neurology* 31, no. 6 (2024): e16267, https://doi.org/10.1111/ene.16267.

13. A. Batra, A. M. Barnard, D. J. Lott, et al., "Longitudinal Changes in Cardiac Function in Duchenne Muscular Dystrophy Population as Measured by Magnetic Resonance Imaging," *BMC Cardiovascular Disorders* 22, no. 1 (2022): 260, https://doi.org/10.1186/s12872-022-02688-5.

14. S. D. Sunthankar, K. George-Durrett, K. Crum, et al., "Comprehensive Cardiac Magnetic Resonance T1, T2, and Extracellular Volume Mapping to Define Duchenne Cardiomyopathy," *Journal of Cardiovascular Magnetic Resonance* 25, no. 1 (2023): 44, https://doi.org/10.1186/s12968-023-00951-y.

15. D. Duboc, C. Meune, G. Lerebours, J. Y. Devaux, G. Vaksmann, and H. M. Bécane, "Effect of Perindopril on the Onset and Progression of Left Ventricular Dysfunction in Duchenne Muscular Dystrophy," *Journal of the American College of Cardiology* 45, no. 6 (2005): 855–857.

16. J. P. Bourke, T. Bueser, and R. Quinlivan, "Interventions for Preventing and Treating Cardiac Complications in Duchenne and Becker Muscular Dystrophy and X-Linked Dilated Cardiomyopathy," *Cochrane Database of Systematic Reviews* 10, no. 10 (2018): CD009068, https://doi.org/10.1002/14651858.CD009068.pub3.

17. N. Ferizovic, J. Summers, I. B. O. de Zárate, et al., "Prognostic Indicators of Disease Progression in Duchenne Muscular Dystrophy: A Literature Review and Evidence Synthesis," *PLoS One* 17, no. 3 (2022): e0265879, https://doi.org/10.1371/journal.pone.0265879.

18. C. Ramaciotti, L. C. Heistein, M. Coursey, et al., "Left Ventricular Function and Response to Enalapril in Patients With Duchenne Muscular Dystrophy During the Second Decade of Life," *American Journal of Cardiology* 98, no. 6 (2006): 825–827.

19. L. Viollet, P. T. Thrush, K. M. Flanigan, J. R. Mendell, and H. D. Allen, "Effects of Angiotensin-Converting Enzyme Inhibitors and/or Beta Blockers on the Cardiomyopathy in Duchenne Muscular Dystrophy," *American Journal of Cardiology* 110, no. 1 (2012): 98–102, https://doi.org/10.1016/j.amjcard.2012.02.064.

20. B. El-Aloul, L. Altamirano-Diaz, E. Zapata-Aldana, et al., "Pharmacological Therapy for the Prevention and Management of Cardiomyopathy in Duchenne Muscular Dystrophy: A Systematic Review," *Neuromuscular Disorders* 27, no. 1 (2017): 4–14, https://doi.org/10.1016/j.nmd.2016.09.019.

21. J. P. Bourke, G. Watson, F. Muntoni, for the DMD Heart Protection study group, et al., "Randomised Placebo-Controlled Trial of Combination ACE Inhibitor and Beta-Blocker Therapy to Prevent Cardiomyopathy in Children With Duchenne Muscular Dystrophy? (DMD Heart Protection Study): A Protocol Study," *BMJ Open* 19;8(12) (2018): e022572, https://doi.org/10.1136/bmjopen-2018-022572.

22. J. P. Bourke, G. Watson, S. Spinty, DMD Heart Study Group, et al., "Preventing Cardiomyopathy in DMD: A Randomized Placebo-Controlled Drug Trial," *Neurology. Clinical Practice* 11, no. 5 (2021): e661–e668, https://doi.org/10.1212/CPJ.0000000000001023.

23. S. Dittrich, E. Graf, R. Trollmann, German Competence Network for Congenital Heart Defects and the Treat-NMD Neuromuscular Network Investigators list of additional local Investigators and co-workers of the German Competence Network for Congenital Heart Defects and the Treat-NMD Neuromuscular Network, et al., "Effect and Safety of

Treatment With ACE-Inhibitor Enalapril and β-Blocker Metoprolol on the Onset of Left Ventricular Dysfunction in Duchenne Muscular Dystrophy - a Randomized, Double-Blind, Placebo-Controlled Trial," *Orphanet Journal of Rare Diseases* 14, no. 1 (2019): 105, https://doi.org/10.1186/s13023-019-1066-9.

24. L. W. Markham, K. Kinnett, B. L. Wong, D. Woodrow Benson, and L. H. Cripe, "Corticosteroid Treatment Retards Development of Ventricular Dysfunction in Duchenne Muscular Dystrophy," *Neuromuscular Disorders* 18, no. 5 (2008): 365–370, https://doi.org/10.1016/j.nmd.2008.03.002.

25. S. M. Szabo, R. M. Salhany, A. Deighton, M. Harwood, J. Mah, and K. L. Gooch, "The Clinical Course of Duchenne Muscular Dystrophy in the Corticosteroid Treatment Era: A Systematic Literature Review," *Orphanet Journal of Rare Diseases* 16, no. 1 (2021): 237, https://doi.org/10.1186/s13023-021-01862-w.

26. C. M. McDonald, O. H. Mayer, K. N. Hor, et al., "Functional and Clinical Outcomes Associated With Steroid Treatment Among Non-ambulatory Patients With Duchenne Muscular Dystrophy," *Journal of Neuromuscular Diseases* 10, no. 1 (2022): 67–79, https://doi.org/10.3233/JND-221575.

27. B. J. Barber, J. G. Andrews, Z. Lu, et al., "Oral Corticosteroids and Onset of Cardiomyopathy in Duchenne Muscular Dystrophy," *Journal of Pediatrics* 163, no. 4 (2013): 1080–1084, https://doi.org/10.1016/j.jpeds.2013.05.060.

28. D. Duboc, C. Meune, B. Pierre, et al., "Perindopril Preventive Treatment on Mortality in Duchenne Muscular Dystrophy: 10 years' Follow-Up," *American Heart Journal* 154, no. 3 (2007): 596–602, https://doi.org/10.1016/j.ahj.2007.05.014.

29. A. Lechner, J. J. Herzig, J. G. Kientsch, et al., "Cardiomyopathy as Cause of Death in Duchenne Muscular Dystrophy: A Longitudinal Observational Study," *ERJ Open Research* 9, no. 5 (2023): 2023, https://doi.org/10.1183/23120541.00176-2023.

30. J. Kipke, D. J. Birnkrant, J. B. Jin, A. Aneja, and R. C. Bahler, "A Systematic Review of Pharmacologic Therapies for the Cardiomyopathy of Duchenne Muscular Dystrophy," *Pediatric Pulmonology* 56, no. 4 (2021): 782–795, https://doi.org/10.1002/ppul.25261.

31. R. Porcher, I. Desguerre, H. Amthor, et al., "Association Between Prophylactic Angiotensin-Converting Enzyme Inhibitors and Overall Survival in Duchenne Muscular Dystrophy-Analysis of Registry Data," *European Heart Journal* 42, no. 20 (2021): 1976–1984, https://doi.org/10.1093/eurheartj/ehab054.

32. S. V. Raman, K. N. Hor, W. Mazur, et al., "Eplerenone for Early Cardiomyopathy in Duchenne Muscular Dystrophy: A Randomised, Double-Blind, Placebo-Controlled Trial," *Lancet Neurology* 14, no. 2 (2015): 153–161, https://doi.org/10.1016/S1474-4422(14)70318-7.

33. S. V. Raman, K. N. Hor, W. Mazur, et al., "Eplerenone for Early Cardiomyopathy in Duchenne Muscular Dystrophy: Results of a Two-Year Open-Label Extension Trial," *Orphanet Journal of Rare Diseases* 12, no. 1 (2017): 39, https://doi.org/10.1186/s13023-017-0590-8.

34. S. V. Raman, K. N. Hor, W. Mazur, et al., "Stabilization of Early Duchenne Cardiomyopathy With Aldosterone Inhibition: Results of the Multi-Center AIDMD Trial," *Journal of the American Heart Association* 8, no. 19 (2019): e013501, https://doi.org/10.1161/JAHA.119.013501.

35. S. Mavrogeni, A. Papavasiliou, K. Giannakopoulou, et al., "Oedema-Fibrosis in Duchenne Muscular Dystrophy: Role of Cardiovascular Magnetic Resonance Imaging," *European Journal of Clinical Investigation* 47, no. 12 (2017): e12843, https://doi.org/10.1111/eci.12843.

36. S. Lee, M. Lee, and K. N. Hor, "The Role of Imaging in Characterizing the Cardiac Natural History of Duchenne Muscular Dystrophy," *Pediatric Pulmonology* 56, no. 4 (2021): 766–781, https://doi.org/10.1002/ppul.25227.

37. M. C. Silva, T. A. Magalahaes, Z. M. Meira, et al., "Myocardial Fibrosis Progression in Duchenne and Becker Muscular Dystrophy: A Randomised Clinical Trial," *JAMA Cardiol* 2, no. 2 (2017): 190–199, https://doi.org/10.1001/jamacardio.2016.4801.