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ORIGINAL RESEARCH ARTICLE

# Heart rate kinetics during standard cardiopulmonary exercise testing in heart transplant recipients: a longitudinal study

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## Abstract

**Aims** Heart transplantation (HTx) results in complete autonomic denervation of the donor heart, causing resting tachycardia and abnormal heart rate (HR) responses to exercise. We determined the time course of suggestive cardiac reinnervation post HTx and investigated its clinical significance.

**Methods and results** Heart rate kinetics during standard cardiopulmonary exercise testing at 2.5–5 years after HTx was assessed in 58 patients. According to their HR increase 30 s after exercise onset, HTx recipients were classified as denervated (slow responders: $<$5 beats per minute [b.p.m.]) or potentially reinnervated (fast responders: $\geq$5 b.p.m.). Additionally, in 30 patients, longitudinal changes of maximal oxygen consumption and HR kinetics were assessed during the first 15 post-operative years. At 2.5–5 years post HTx, 38% of our study population was potentially reinnervated. Fast responders were significantly younger (41 ± 15 years) than slow responders (53 ± 13 years, $P$ = 0.003) but did not differ with regard to donor age, immunosuppressive regime, cardiovascular risk factors, endomyocardial biopsy, or vasculopathy parameters. While HR reserve (56 ± 20 vs. 39 ± 15 b.p.m., $P$ = 0.002) and HR recovery after 60 s (15 ± 11 vs. 5 ± 6 b.p.m., $P < 0.001$) were greater in fast responders, resting HR, peak HR of predicted, and peak oxygen consumption of predicted were comparable.

**Conclusions** Signs of reinnervation occurred mainly in younger patients. Maximal oxygen consumption was independent of HR kinetics.

**Keywords** Heart transplantation; Heart rate; Heart rate recovery; Exercise testing; Exercise capacity; Reinnervation

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## Introduction

Heart transplantation (HTx) causes complete surgical denervation of the donor heart, resulting in resting tachycardia, a slower increase in heart rate (HR) at the onset of exercise, a blunted chronotropic response to exercise in general, and a slower decline in HR after exercise, compared with healthy subjects.[1] There is substantial evidence that early after HTx (starting at approximately 6–18 months post HTx), the gradual increase in HR observed during exercise can be attributed to circulating catecholamines[1] and partial sympathetic reinnervation of the cardiac allograft,[2,3] whereas the occurrence of parasympathetic reinnervation occurs later and not in all patients.[4–7] Various invasive procedures to determine catecholamine concentrations and non-invasive methods like $^{123}$I-meta-iodobenzylguanidine uptake[8,9] and positron emission tomography (PET) with the catecholamine analogue C-11 hydroxyephedrine have been applied to evaluate the reinnervation process by the autonomic nervous system.[10,11] Cardiopulmonary exercise testing (CPET), however, may provide a simple and non-invasive option to assess reinnervation post HTx.[4,5,12] Overall, the exact timing and extent of cardiac allograft reinnervation by the autonomic nervous system are unclear, and serial assessment of HR parameters may,

therefore, provide information on the time course of autonomic reinnervation. Furthermore, two studies in paediatric HTx patients reported absence of suggestive reinnervation based on HR recovery[13] or HR variability[14] to be associated with worse outcome. Two studies in adult HTx patients have found resting HR $>$ 100 b.p.m.[15] and HR $>$ 90 b.p.m.[16] to be associated with higher mortality.

Accordingly, the aims of this study were to (i) determine the prevalence of presumed cardiac autonomic reinnervation, (ii) compare HR parameters and exercise capacity between denervated and potentially reinnervated subjects, (iii) longitudinally assess the time course of suggestive cardiac allograft reinnervation, and (iv) assess a potential difference in outcome of denervated and potentially reinnervated patients.

# Methods

We conducted a retrospective analysis of cross-sectional and longitudinal data on HR kinetics during CPET in patients after HTx. Patients were recruited from the heart transplant outpatient clinic at the Bern University Hospital. The study complied with the Declaration of Helsinki and was approved by the Cantonal Ethics Committee of Bern.

## Study population

All HTx patients who had annual follow-up examinations between February 2003 and June 2016 at the heart transplant outpatient clinic at the Bern University Hospital were assessed for eligibility. Exclusion criteria with corresponding number of patients are shown in Supporting Information, *Figure S1*.

For the cross-sectional analysis, patients with at least one exercise test between 2.5 and 5 years post HTx were included and categorized into two groups according to the classification of HR response to exercise defined in Section "Classification of heart rate response to exercise." If a patient had more than one exercise test during this time, only the first exercise test was included. Cross-sectional groups were compared with regard to parameters of HR kinetics and exercise capacity as outlined in Section "Cardiopulmonary exercise testing." For the longitudinal analysis, we grouped exercise tests into three time bins as follows: Bin 1 from 0 to $<$2.5 years post HTx; Bin 2 from 2.5 to $<$5 years post HTx; and Bin 3 from 5 to $<$15 years post HTx. Only patients with at least one exercise test in every time bin were included and categorized according to the criteria defined in Section "Classification of heart rate response to exercise." Within these groups, changes in parameters of HR kinetics and exercise capacity were analysed over time. If a patient had more than one exercise test in a specified time bin, only the first exercise test in Bin 1 and Bin 2 and only the last exercise test in Bin 3 were included, unless HR parameters were not available for this test, in which case another test of the same time bin was chosen.

The subsequently described procedures were all performed within 1 week of the respective CPET (cross-sectional and longitudinal).

## Data collection and risk factor management

All clinical data, including recipient and donor characteristics, and concomitant medication were retrieved by retrospective review of patient databases. In case of high-risk cytomegalovirus serologic status (i.e. donor positive and recipient negative), HTx recipients received ganciclovir or valganciclovir cytomegalovirus prophylaxis adapted to individual kidney function for 3–6 months. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg[17] or ongoing medical antihypertensive therapy.

## Blood testing

Blood samples were collected to assess cardiovascular risk factors (i.e. blood lipid profile and glycated haemoglobin) and renal function. Hypercholesterolaemia and diabetes mellitus were defined based on current guidelines[18] or ongoing medical therapy. Renal function was assessed by an estimate of the glomerular filtration rate calculated by the Chronic Kidney Disease Epidemiology Collaboration study.[19]

## Endomyocardial biopsy

Protocol endomyocardial biopsy (EMB) specimens were performed weekly during the first post-operative month. Up to 1 year, the intervals were extended depending on the individual clinical course. Thereafter, in the absence of signs of acute rejection or major change in immunosuppressive medication, yearly EMBs were performed. EMB specimens were graded according to the 2004 International Society for Heart and Lung Transplantation (ISHLT) classification for acute cellular rejection as 0R, 1R, 2R, and 3R.[20] Severe total rejection score is defined as number of acute cellular rejection $\geq$ 2R divided by the total number of biopsies. Antibody-mediated rejection was diagnosed in the EMB specimens according to standardized histopathologic signs[21] and, if indicated, confirmed with immunohistochemistry. No antibody-mediated rejection-positive EMBs confirmed with immunohistochemistry or allograft rejections with haemodynamic compromise were observed.


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### Coronary angiography

Cardiac allograft vasculopathy (CAV) was assessed retrospectively and graded visually according to the current nomenclature of the ISHLT (ISHLT-CAV 0–3).[22] Moreover, the presence of CAV was defined as a status ≥ISHLT-CAV 1.[22]

### Transthoracic echocardiography

Routine transthoracic echocardiography was performed to assess cardiac chamber morphology, systolic and diastolic left ventricular function in particular.[23]

### Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed on an electronically braked cycling ergometer using a continuous, symptom-limited exercise protocol. Following a 3 min resting phase, a warm-up was performed at a constant load of 20 watts (W) for another 3 min. Then, a ramp protocol at a cadence of 60–70 revolutions per minute (r.p.m.) with a work rate increment of either 10, 15, or 20 W/min was started, based on the patient's previously measured or estimated physical fitness, with the goal of reaching exhaustion within 8–12 min. After reaching exhaustion, patients continued cycling at 25 W with 20–30 r.p.m. for approximately 1 min. Thereafter, patients sat quietly for another 2 min. Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and minute ventilation ($\dot{V}_E$) were measured using a breath-by-breath gas analyser (Jaeger Oxycon Pro, CareFusion GmbH, Hoechberg, Germany). HR was monitored continuously before, during, and after exercise using a 12-lead electrocardiogram and then stored as breath-by-breath data. Examples of CPETs are shown in Supporting Information, *Figure S2*.

### Data processing

Data processing in MATLAB is described in detail in the Supporting Information.

### Classification of heart rate response to exercise

After visual inspection of our data and in accordance with existing literature,[1,3,24] patients were divided into two groups according to their HR response at the onset of exercise. If at 30 s after the onset of warm-up exercise at 20 W HR had increased by 5 or more b.p.m. from resting HR, patients were classified as fast responders and potentially reinnervated; otherwise, patients were classified as slow responders and likely to still be denervated. This classification was based on a study who used the acute response to tyramine injection to identify HTx patients without sympathetic reinnervation when the increase in HR was $< 5$ b.p.m.[3] The time window of 30 s was chosen because after 30 s from onset of exercise, plasma noradrenaline levels were found to increase in denervated HTx patients.[1] To be classified as fast responder, at least one available exercise test within the time period of 0–15 years post HTx had to show an HR increase of 5 or more b.p.m. Consequently, all exercise tests of such patients appear in the group of fast responders.

### Statistical analyses

Data were visually checked for normal distribution with q–q plots. Results are presented as mean ± standard deviation or median (interquartile range) as appropriate. HR kinetics were compared cross-sectionally (i.e. between groups) using either Welch's *t*-test, Mann–Whitney *U* test, or $\chi^2$/Fisher's exact test, as appropriate. In the case of significant between-group differences in patient age, subgroup analyses were performed with subgroups comparable with regard to age. Longitudinal between-group and within-group changes were analysed by two-way ANOVA. A $P$-value $< 0.05$ was considered statistically significant. Statistical analyses were performed with R software (version 3.3.0, The R Foundation for Statistical Computing).

## Results

### Study population

One hundred and twenty-nine HTx patients met the inclusion criteria (Supporting Information, *Figure S1*). For the cross-sectional analysis, 58 HTx with an exercise test between 2.5 and 5 years after transplantation were included. Of these, 38% were classified as fast responders and the other 62% as slow responders. Detailed patient characteristics are shown in *Table 1*. HR-modulating medication did not differ between groups, and cardiovascular risk factors were comparable, except for total cholesterol, which was higher in slow responders. Moreover, graft rejections requiring treatment and CAV were not significantly different between fast responders and slow responders. Otherwise, the groups were comparable except for age at HTx ($P$ = 0.003), with fast responders being on average 12 years younger. During the follow-up period, death or retransplantation occurred in four patients of the fast-responder group (18%) and six patients in the slow-responder group (17%, $\chi^2$ $P$-value=0.901).

### Heart rate kinetics

Because our groups of fast responders and slow responders significantly differed with regard to age (by 12 years), we compared HR kinetics and exercise capacity of these two groups

**Table 1** Patient characteristics

| | Fast responders ($n$ = 22, 38%) | Slow responders ($n$ = 36, 62%) | $P$-value |
|---|---|---|---|
| Recipient age at HTx (years) | 37.2 ± 15.1 | 49.4 ± 12.4 | 0.003 |
| Recipient sex (male) | 16 (73%) | 27 (75%) | 0.841 |
| Donor age at HTx (years) | 39.6 ± 12.5 | 42.3 ± 13.3 | 0.451 |
| Donor sex (male) | 18 (82%) | 27 (75%) | 0.546 |
| ICM pre HTx | 3 (14%) | 11 (31%) | 0.144 |
| CMV infection | 6 (27%) | 13 (36%) | 0.486 |
| Cardiovascular risk factors[a] | | | |
| Active smoker | 2 (9%) | 3 (8%) | 1.000 |
| Hypertension | 15 (68%) | 31 (86%) | 0.102 |
| Systolic BP (mmHg) | 122.8 ± 15.9 | 131.4 ± 17.5 | 0.064 |
| Diastolic BP (mmHg) | 75.8 ± 10.4 | 80.4 ± 10.1 | 0.100 |
| Pulse pressure (mmHg) | 47.0 ± 17.0 | 51.0 ± 16.0 | 0.369 |
| Mean arterial pressure (mmHg) | 91.5 ± 9.5 | 97.4 ± 10.7 | 0.036 |
| Diabetes | 4 (18%) | 3 (8%) | 0.409 |
| HbA1c (%) | 5.6 ± 0.7 | 5.8 ± 0.5 | 0.458 |
| Total cholesterol (mmol/L) | 3.9 ± 0.9 | 5.0 ± 1.2 | 0.002 |
| LDL cholesterol (mmol/L) | 2.2 ± 0.9 | 2.7 ± 0.8 | 0.088 |
| eGFR (mL/min)[a] | 69.6 ± 31.9 | 63.2 ± 26.7 | 0.431 |
| Endomyocardial biopsy | | | |
| Patients with EMB ≥ 2R[b] | 14 (64%) | 18 (50%) | 0.311 |
| Severe TRS ≥ 2R[a] | 0.057 (0.117) | 0.017 (0.090) | 0.166 |
| Cardiac allograft vasculopathy[a] | | | 0.175 |
| 0 | 15 (68%) | 18 (50%) | |
| ≥1 | 7 (32%) | 18 (50%) | |
| Echocardiography[a] | | | |
| LVEF (%) | 64.0 ± 8.6 | 61.7 ± 6.4 | 0.270 |
| e' septal (cm/s) | 8.6 ± 2.4 | 8.1 ± 1.8 | 0.480 |
| Medication | | | |
| Beta-blockers | 3 (14%) | 6 (17%) | 1.000 |
| % of max. dose | 38 ± 22 | 40 ± 35 | 0.922 |
| Calcium channel blockers | 5 (23%) | 9 (25%) | 0.844 |
| % of max. dose | 57 ± 15 | 59 ± 12 | 0.729 |

BP, blood pressure; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; EMB, endomyocardial biopsy; e' septal, peak early diastolic mitral annulus velocity at septal basal region; HbA1c, glycated haemoglobin; HTx, heart transplantation; ICM, ischaemic cardiomyopathy; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; TRS, total rejection score.
Data are presented as mean ± standard deviation, median (interquartile range), or $n$ (%).
[a]At last follow-up.
[b]Until 2.5 years post HTx.

(*Table 2*) as well as of two age-matched groups (Supporting Information, *Table S1*), including patients aged maximally 45 years at HTx, resulting in a group of 16 fast responders (57%) and 12 slow responders (43%). As per definition, HR increase during the first 30 s was greater in fast responders compared with slow responders of both age groups (7 ± 7 vs. 1 ± 1 b.p.m., $P$ = 0.001, in total group and 8 ± 7 vs. 2 ± 2 b.p.m., $P$ = 0.002, in subgroup). HR response at exercise cessation, namely, HR recovery after 60 s, was also significantly greater in fast responders compared with slow responders (15 ± 11 vs. 5 ± 6 b.p.m., $P < 0.001$, in total group and 19 ± 10 vs. 6 ± 9 b.p.m., $P$ = 0.002, in subgroup). Likewise, HR reserve was greater in fast responders (56 ± 20 vs. 39 ± 15 b.p.m., $P$ = 0.002, in total group and 62 ± 19 vs. 46 ± 19 b.p.m., $P$ = 0.044, in subgroup). Because of the strong dependence on age, peak HR was higher in fast responders of the total group (146 ± 22 vs. 131 ± 19 b.p.m., $P$ = 0.016) but not in the age-matched subgroup (152 ± 19 vs. 142 ± 19 b.p.m., $P$ = 0.193). Consequently, peak HR of predicted was comparable between fast responders and slow responders in the total group and the age-matched subgroup. Resting HR was comparable between fast responders and slow responders of both the total group (90 ± 14 vs. 93 ± 11 b.p.m., $P$ = 0.410) and the subgroup (89 ± 15 vs. 97 ± 10 b.p.m., $P$ = 0.129).

ANOVAs for longitudinal data including 30 patients with sufficient longitudinal data showed a trend in the group × time interaction for HR response to exercise onset (i.e. HR at 30 s after exercise onset, $P$ = 0.054) with a gradual minimal increase in slow responders and an increase from 1 to 3 years post HTx and decline again to year 8 in fast responders (*Figure 1A*). HR recovery at all measured time points (i.e. 30, 60, and 120 s after cessation of exercise) differed between groups and over time (all $P \leq 0.007$; *Figure 1B*), with higher values in the fast-responder group and increasing values with time. Resting HR declined significantly over time and was lower in fast responders compared with slow

**Table 2** Cardiopulmonary exercise testing data from the first test within 2.5–5 years post HTx

| Parameters | Fast responders ($n$ = 22) | Slow responders ($n$ = 36) | $P$-value |
|---|---|---|---|
| Recipient age at test (years) | 40.5 ± 15.1 | 52.7 ± 12.5 | 0.003 |
| Time post HTx (years) | 3.3 ± 0.6 | 3.3 ± 0.5 | 0.653 |
| Weight (kg) | 73.1 ± 16.4 | 72.2 ± 12.5 | 0.831 |
| Height (cm) | 172.2 ± 8.1 | 170.3 ± 6.3 | 0.368 |
| BMI (kg/m$^2$) | 24.5 ± 5.0 | 24.7 ± 3.2 | 0.867 |
| Resting HR (b.p.m.) | 89.6 ± 14.1 | 92.5 ± 11.1 | 0.410 |
| HR increase after 30 s of exercise (b.p.m.) | 6.8 ± 6.5[a] | 1.2 ± 1.4[b] | 0.001 |
| Peak HR (b.p.m.) | 145.7 ± 21.7[c] | 131.0 ± 18.7[d] | 0.016 |
| Percentage of predicted peak HR (%) | 81.1 ± 9.2[c] | 78.2 ± 10.7[d] | 0.301 |
| HR reserve (b.p.m.) | 56.0 ± 20.4[c] | 38.6 ± 14.9[d] | 0.002 |
| HR recovery 30 s (b.p.m.) | 7.7 ± 6.8[a] | 2.2 ± 4.5[d] | 0.003 |
| HR recovery 60 s (b.p.m.) | 14.9 ± 10.9[a] | 4.6 ± 6.2[d] | <0.001 |
| HR recovery 120 s (b.p.m.) | 25.1 ± 14.6[e] | 9.3 ± 9.4[b] | <0.001 |
| $\dot{V}O_2$ peak (mL/min/kg) | 23.3 ± 7.9[c] | 19.9 ± 5.1[f] | 0.088 |
| Percentage of predicted $\dot{V}O_2$ peak (%) | 68.1 ± 14.9[c] | 68.7 ± 17.2[f] | 0.886 |
| $\dot{V}_E$ / $\dot{V}CO_2$ slope | 35.9 ± 10.5 | 36.7 ± 6.4[f] | 0.772 |

BMI, body mass index; HR, heart rate; HTx, heart transplantation.
Data are presented as mean ± standard deviation.
[a]$n$ = 20.
[b]$n$ = 30.
[c]$n$ = 21.
[d]$n$ = 31.
[e]$n$ = 19.
[f]$n$ = 34.

responders (both $P < 0.02$; *Figure 1C*). Peak HR of predicted increased slightly ($P$ = 0.047) over time with no difference between groups (*Figure 1D*). All available data for HR increase at 30 s after exercise onset and HR recovery at 60 s after exercise cessation are shown in Supporting Information, *Figure S3*, top and middle panels.

## Exercise capacity

Because of the younger age of fast responders, absolute exercise capacity tended to be higher in fast responders than slow responders (23 ± 8 vs. 20 ± 5 mL/min/kg, $P$ = 0.088; *Table 2*). However, relative exercise capacity in terms of percentage of age-predicted, sex-predicted, weight-predicted, and height-predicted $\dot{V}O_2$ peak (68 ± 15 vs. 69 ± 17%, $P$ = 0.886) were comparable between fast responders and slow responders in both the total group and also the ≤45 years subgroup. Likewise, there was no difference between groups with regard to $\dot{V}_E$ / $\dot{V}CO_2$ slope (*Table 2*). $\dot{V}O_2$ peak of predicted increased slightly by trend in both groups, with no difference between groups ($P$ = 0.077; *Figure 1E*). All available data of peak oxygen uptake of predicted are shown in Supporting Information, *Figure S3*, bottom panel.

## Clinical parameters

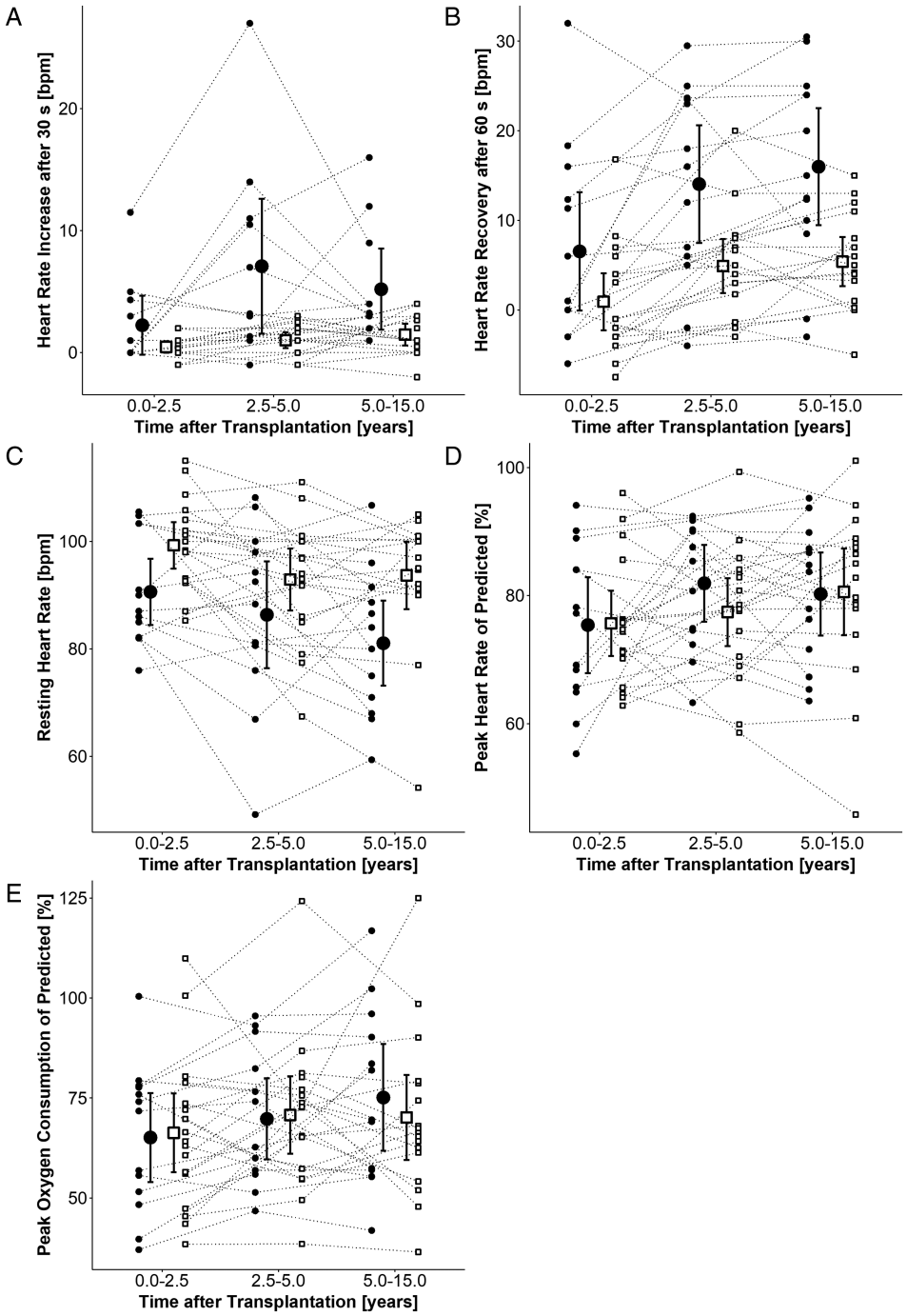
Mean arterial blood pressure was lower in fast responders compared with slow responders (92 vs. 97 mmHg; *Table 1*); however, this difference disappeared after correcting for age. Total cholesterol was also lower in the potentially reinnervated patients (3.9 vs. 5.0 mmol/L); this difference was reduced after adjustment for age ($P$ = 0.014). There were no differences with regard to donor age, aetiology, complications, cardiac medication, immunosuppressive regime, and cardiovascular risk factors.

# Discussion

In our cross-sectional population of HTx patients, 38% showed signs of autonomic reinnervation of the cardiac allograft. These patients were significantly younger than those with poor HR kinetic responses to exercise but were not different with regard to donor age, immunosuppressive regime, cardiovascular risk factors, EMB, or vasculopathy parameters, nor outcome. Patients with faster HR increase at the onset of exercise had greater HR reserve and faster HR recovery after exhaustion. Congruent with previous studies,[3,26] we found a moderately reduced exercise capacity (68% of predicted peak VO2) in our HTx patients, with no evidence of better exercise capacity in those who were supposedly reinnervated.[27] Serial assessment of HR parameters in presumably reinnervated patients revealed that reinnervation occurred within the first 3 years after HTx with no further improvement thereafter.

Heart rate responses during and after exercise have been associated with functional efferent reinnervation of the sinus node in patients after HTx.[3,12] The occurrence of sympathetic reinnervation of the ventricles as well as the sinus node has been investigated by invasive and non-invasive techniques

**Figure 1** (A) Heart rate increase after 30 s of exercise onset, (B) heart rate recovery 60 s after cessation of maximal exercise, (C) resting heart rate in sitting position before the start of the exercise test, (D) peak heart rate at maximal exercise expressed as percentage of age-predicted maximal heart rate [peak heart rate/(220 – age)], and (E) peak oxygen consumption expressed as percentage of height-predicted, weight-predicted, sex-predicted, and age-predicted peak oxygen consumption according to Wasserman[25] by groups. Mean values and 95% confidence intervals are presented for the fast responders (filled circles) and slow responders (empty squares) for selected time points. Small filled circles and empty squares represent individual subjects with fast responders located on the left and slow responders located on the right of mean values.





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at several time points after HTx.[2–4,28,29] Comparable with our results, between approximately 2 and 4 years post HTx, the prevalence of sympathetic reinnervation has been reported to be around 55% based on PET or $^{123}$I-meta-iodobenzylguanidine uptake.[2,29] The mean age at HTx in our population was 12 years younger in the supposedly reinnervated group compared with the supposedly denervated group, emphasizing the importance of young age for autonomic reinnervation. This is in accordance to a PET imaging study that found recipient age to be the only variable related to the degree of sympathetic reinnervation.[30] Similarly, a small study in paediatric, adolescent, and adult HTx patients found that reinnervation decreased with increasing age at HTx and was not influenced by the age of the donor heart.[27] Studies in paediatric HTx patients have found suggestive reinnervation in 57%[14] and at least 75% (percentage estimated from shown data),[13] supporting the argument that reinnervation is more likely at a younger recipient age. On the other hand, Bengel *et al*. found a stronger inverse relationship of reinnervation with donor age than recipient age.[11]

In the present study, we found an improvement in the HR increase at the onset of exercise within 3 years after HTx with no further improvement thereafter in the fast-responder group. This improvement was, however, not paralleled by any other parameter in longitudinal assessment, except for HR recovery. This is in accordance to a study by Giardini *et al*., who retrospectively analysed serial peak exercise tests in paediatric Tx recipients and found that HR recovery normalized at approximately 6 years after HTx.[13] Similarly, a study by Cornelissen *et al*. found a decrease in resting HR and increase in HR variability between approximately the first to the fourth year after HTx.[31] On the other hand, Singh *et al*. reported that HR recovery after maximal exercise testing in a paediatric HTx population progressively improved with time since transplantation.[32] In addition, resting HR decreased, and the percentage of age-predicted peak HR increased in the longitudinal exercise assessment of their study. We attribute the lack of longitudinal improvement in other HR parameters to the older age of our population and the large intra-subject changes appearing over time in our study. Likewise, Givertz *et al*.[26] also found no increase in HR reserve in 57 adult HTx patients followed over the first 5 years after HTx. HR reserve is possibly not a suitable parameter for assessment of reinnervation as peak HR strongly depends on test duration and circulating catecholamine levels. Interestingly, we found large fluctuations in all measured HR kinetic parameters from year to year (Supporting Information, *Figure S3*, top and middle panels). Whether these fluctuations over time are secondary to changes in the degree of reinnervation is not known and has, to our knowledge, not been studied.

In previous studies of HTx recipients, exercise capacity improved compared with the pre-transplant state within 1 year after HTx with no further improvement thereafter but remained below levels observed in healthy people.[26,33,34] In a review by Nytrøen and Gullestad, the percentage of predicted $\dot{V}O_2$ peak ranged from 50% to 70% in most studies,[35] which coincides with the value observed in the present study (68%). However, it should be noted that our patients covered a wide range of predicted $\dot{V}O_2$ peak, namely, from 30% to 125% (Supporting Information, *Figure S3*, bottom panel), and relatively large fluctuations between yearly tests were present. Bengel *et al*. showed that better chronotropic and inotropic competence was associated with better exercise capacity in reinnervated patients.[36] Similarly, in a study on paediatric HTx patients, reinnervation as assessed by increased HR variability was associated with improved peak oxygen uptake.[14] We did not find an association between relative exercise capacity and the state of reinnervation; however, this may have been due to other factors having a relevant impact on exercise capacity in our patient population. Several groups found that skeletal muscle abnormalities of advanced heart failure last for indefinite time after HTx and that these abnormalities may limit exercise performance in these patients.[37–40] Other studies have also found chronotropic incompetence not to be a limiting factor for exercise capacity in HTx[41,42] and chronotropic improvement not forcibly to be associated with improvement in exercise capacity.[26,27,43]

## Limitations

The first limitation concerns the retrospective design of this study. The mean exercise capacity found in our HTx population may overestimate the true exercise capacity of all patients transplanted in Bern as patients were likely to refuse testing when they were in a very unstable state of health.

Further, the chosen criterion to differentiate supposedly reinnervated from denervated patients was, though based on existing studies, somewhat arbitrary. The two groups classify different degrees of reinnervation rather than absent and present reinnervation and cannot distinguish between sympathetic and parasympathetic reinnervation of the sinus node. Lastly, the rather small sample size could have limited statistical power to detect any longitudinal changes in HR parameters and clinical outcome.

The strength of this study, however, is the detailed analysis of serial exercise testing in the same patients with a long-time follow-up using raw CPET data analysed with automated algorithms. Longitudinal changes revealed large individual fluctuations in HR parameters over time, which have not been shown in previous studies that have shown mean values with standard deviations over time only.[44] This indicates that reinnervation may fluctuate rather than reach a constant level.

## Conclusions

Three years after HTx, HR responses during CPET and exercise capacity were reduced compared with predicted values for healthy people. Our longitudinal data suggest that functional reinnervation of the cardiac allograft, especially of the sympathetic nervous system, mainly occurs within 3 years after HTx with no significant improvements thereafter mainly in those with younger age at transplantation. Improved HR kinetics achieved by reinnervation does not seem to be pivotal for exercise capacity in this patient population.

## Acknowledgement

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## Conflict of interest

O.S., L.D.T., D.H. P.M., V.S., M.W., and P.E. declare that they have no conflict of interest.

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The authors did not receive any funding for this work.

## Author contributions

O.S. designed the study, collected and analysed the data, and wrote the manuscript. L.D.T. and P.M. designed the study, collected the data, and critically revised and approved the manuscript. D.H. designed the study, analysed the data, and critically revised and approved manuscript. V.S. collected the data and critically revised and approved manuscript. M. W. designed the study and critically revised and approved the manuscript. P.E. designed the study, analysed the data, and wrote the manuscript.

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** Flow chart of patient exclusion process. n = number of patients, $n_{Test}$ = number of exercise tests.
**Figure S2** Cardiopulmonary exercise tests from one healthy person with normal innervation (A) and three heart transplant recipients with no reinnervation (B), assumed sympathetic reinnervation (C) and assumed sympathetic and parasympathetic reinnervation (D). Purple: heart rate (HR); green: workload (Watt); red: carbon dioxide production (V'CO2); blue: oxygen consumption (V'O2).
**Figure S3** All available data for heart rate increase after 30 s of exercise onset (top panel), heart rate recovery 60 s after cessation of maximal exercise (middle panel), and peak oxygen consumption expressed as percentage of height-, weight-, sex-, and age-predicted peak oxygen consumption according to Wasserman (bottom panel). Filled circles represent fast responders and empty squares represent slow responders. The horizontal line in panel A represent the cut-off of 5 beats used for the group classification.
**Table S1** Heart rate parameters from first CPET within 2.5–5 years post HTx for patients ≤45 years at HTx.

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