Functional evidence of low-pressure cardiopulmonary baroreceptor reinnervation one year after heart transplantation

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Abstract

Purpose

Heart transplantation (HTx) implies denervation of afferent neural connections. Reinnervation of low-pressure cardiopulmonary baroreceptors might impact the development and treatment of hypertension, but little is known of its occurrence. The present prospective study investigated possible afferent reinnervation of low-pressure cardiopulmonary baroreceptors during the first year after heart transplantation.

Methods

A total of 50 heart transplant recipients (HTxRs) were included and were evaluated 7-12 weeks after transplant surgery, with follow-up 6 and 12 months later. In addition, a reference group of 50 healthy control subjects was examined once. Continuous, non-invasive recordings of cardiovascular variables were carried out at supine rest, during 15 minutes of 20^{o} head-up tilt, during Valsalva maneuver and during 1 minute of 30% maximal voluntary handgrip. In addition, routine clinical data including invasive measurements were used in the analyses.

Results

During the first year after HTx: the heart rate (HR) response to 20^{o} head-up tilt partly normalized; a negative relationship between resting mean right atrial pressure and HR tilt response developed; low-frequency variability of the RR-interval and systolic blood pressure at supine rest increased; and the total peripheral resistance response to Valsalva maneuver became stronger.

Conclusion

Functional assessments suggest that afferent reinnervation of low-pressure cardiopulmonary receptors occurs during the first year after heart transplantation, partially restoring reflexively mediated responses to altered cardiac filling.

Keywords

Heart transplantation; autonomic cardiovascular control; denervation; cardiopulmonary receptors

List of abbreviations

AccHEART = Autonomic Cardiovascular Control after Heart Transplantation

CI=Cardiac Index

DBP=Diastolic Blood Pressure

EDVI=End-Diastolic Volume Index

HR=Heart Rate

HTx=Heart Transplantation

HTxRs = Heart Transplant Recipients

LF=Low Frequency Power (of cardiovascular variability)

MAP=Mean Arterial Blood Pressure

RAP=Right Atrial Pressure

RRI=RR-Interval

SBP=Systolic Blood Pressure

TPRI=Total Peripheral Resistance Index

Introduction

Heart transplantation (HTx) remains the preferred treatment for end-stage heart failure (Lund 2018). Normally, the heart is intimately controlled by the autonomic nervous system, ensuring immediate compensatory responses to homeostatic aberrations. However, heart transplantation always results in complete cardiac denervation with loss of both efferent and afferent autonomic connections to the donor heart, resulting in severely impaired cardiovascular reflex responses (Awad et al. 2016). Ample evidence suggests that reinnervation might occur over time. In particular, efferent sympathetic reinnervation of the sinus node has been implicated in several studies (Peiss et al. 1966; Wilson et al. 1991; Estorch et al. 1999; Uberfuhr et al. 2000; Christensen et al. 2020). However, the extent of re-innervation – as well as the functional consequences of both the denervation and the re-innervation processes – remain largely unknown.

It is conceivable that the denervation/reinnervation phenomena are causally implicated in complications commonly experienced by HTx recipients (HTxRs). For instance, a tendency towards hypertension and peripheral vasoconstriction is well known among HTxRs (Idema et al. 1994; Bennett & Ventura 2017), but the underlying mechanisms are still not fully understood. Also, HTxRs have reduced exercise capacity (Nytrøen & Gullestad 2013), and altered cardiovascular reflex adjustments might be a contributing factor.

Recent evidence suggests that denervation of afferent connections from low-pressure cardiopulmonary baroreceptors might contribute to elevated resting blood pressures and total peripheral resistance, as well as blunted orthostatic responses, in recent HTxRs (Nygaard et al. 2019). It is therefore of interest to investigate whether reinnervation of these receptors occurs, and further, how reinnervation might impact hypertension development later after HTx. Also, an improved understanding of the related reflex mechanisms might inform antihypertensive treatment, in particular the usage of diuretics.

Evidence of reinnervation of low-pressure cardiopulmonary baroreceptors can be indirectly provided through functional assessments that engage these receptors and their associated reflex loops. In the present study, the functional assessments included: a) 20^{o} head-up tilt which primarily unload cardiopulmonary receptors without activating arterial baroreceptors (Triedman et al. 1993; Wyller et al. 2007); b) Valsalva-maneuver which activates both low-pressure cardiopulmonary receptors and high-pressure arterial receptors (Goldstein 2001); c) Isometric exercise, which does not activate cardiopulmonary receptors and therefore served as a control experiment (Goldstein 2001).

The aim of the present study was to investigate possible afferent reinnervation of low-pressure cardiopulmonary baroreceptors during the first year after heart transplantation. To the best of our knowledge, this aim has not been specifically addressed by any previous study. We hypothesized that the 20^{o} head-up tilt test as well as the Valsalva-maneuver would provide functional evidence of some degree of reinnervation.

Materials and methods

Design and Participants

This study is part of the AccHEART project (Autonomic Cardiovascular Control after Heart Transplantation; ClinicalTrials ID: NCT01759966), which addresses autonomic denervation and reinnervation in a population-based prospective cohort of HTxRs. Details of the design have been reported elsewhere (Nygaard et al. 2019). AccHEART has been approved by the Regional Committee for Ethics in Medical research.

All HTxRs at the Department of Cardiology, Rikshospitalet, Oslo University Hospital, Norway, between December 2012 and December 2015 were evaluated for enrolment. Inclusion criteria were: a) age 16 to 70 years, and b) transplant surgery performed during the last 7-12 weeks. Exclusion criteria encompassed: a) dysfunction of the allograft; b) comorbid chronic medical conditions; c) ECG abnormalities; d) acute medical complications; and e) non-compliance. In addition, healthy controls with a similar distribution of sex and age were included as a comparison group. Participation was based upon informed consent. Details of the recruitment and inclusion procedure have been reported elsewhere (Nygaard et al. 2019).

Included HTxRs were followed prospectively for a total of three years. The present study reports results from the first (baseline), second (6 months) and third (12 months) appointment. The healthy controls were examined only once.

Autonomic Cardiovascular Assessment

At all appointments, participants attended a standardized investigational program at our study center that included a thorough assessment of autonomic cardiovascular control. The autonomic tests were executed at the same time of day and followed a fixed sequence. All participants were instructed to abstain from tobacco products and caffeine 48 hours prior to attendance, and to fast overnight. They were maintained on immunosuppressive medications,

while all other drugs were paused on the morning of testing. The tests were performed in a calm room with no windows and dimmed light. The ambient temperature was kept around 23 degrees Celsius and the subjects were encouraged not to speak or move during testing.

The autonomic cardiovascular assessment encompassed the following tests: 1) supine rest (5 minutes); 2) 20^{o} head-up tilt (15 minutes); 3) Valsalva maneuver (15 seconds, airway pressure of 40 mmHg, repeated twice); 4) isometric exercise (60 seconds, handgrip of 30% maximal voluntary force, repeated twice). A detailed description is provided elsewhere (Nygaard et al. 2019; Christensen et al. 2020).

The 20^{o} head-up tilt test was specifically designed to primarily unload cardiopulmonary receptors without activating arterial baroreceptors (Triedman et al. 1993; Wyller et al. 2007). A 60^{o} head-up tilt test as well as a controlled breathing test were also included in the assessment program. Results from these tests are not reported in the present paper.

During all tests, subjects were attached to the Task Force Monitor (Model 3040i, CNSystems Medizintechnik, Graz, Austria), a combined hardware and software device for recording non-invasive cardiovascular variables (Fortin et al. 2006). Instantaneous RR-interval (RRI) was obtained from the ECG-signal. Continuous arterial blood pressure (BP) was measured noninvasively by finger plethysmography (Parati et al. 1989). Impedance cardiography with electrodes placed on the neck and lower thorax was used to obtain a continuous recording of the temporal derivate of the transthoracic impedance (dZ/dt) (Denniston et al. 1976). Impedance cardiography has been reported to provide valid flow estimates among HTxRs (Pepke-Zaba et al. 1990). Signals were on-line transferred to the built-in recording computer of the Task Force Monitor, running software for real-time beat-to-beat data acquisition.

Data Analysis

All primary cardiovascular variables were manually inspected, and artifacts (such as non-sinus beats) were removed. Thereafter, heart rate (HR) was calculated from the RRI-signal and stroke volume (SV) and end-diastolic volume (EDV) was calculated from the impedance signal. Cardiac output (CO) was calculated as SV times HR, and total peripheral resistance (TPR) was calculated as mean blood pressure divided by CO. Flow dependent variables were indexed according to body surface area (BSA), estimated from the formula BSA = 0.0235 x height(cm)$^{0.42246}$ x weight(kg)$^{0.51456}$.

For tests where a steady state condition could be assumed (supine rest, 20^{o} head-up tilt), median values of standard cardiovascular variables were calculated from epochs of 240 seconds length in each individual. These epochs were selected to avoid periods of mentally evoked autonomic activity and reflexive autonomic adjustments immediately before and after infliction of orthostatic challenge. In addition, variability analyses (power spectral analyses) of RRI and systolic BP (SBP) were performed in the same epochs, applying an adaptive autoregressive technique (Bianchi et al. 1997). In the present paper, only variability in the low-frequency (LF) band (0.04 – 0.15Hz) are reported, reflecting combined sympathetic and parasympathetic sinus node modulation (RRI-variability) and sympathetic peripheral resistance vessel modulation (SBP-variability), respectively (Task Force 1996; Malpas 2002). The cardiovascular response to orthostatic challenge was defined as the difference (delta values) between tilt values and supine rest values (ie., delta values = tilt values - supine rest values).

For the isometric exercise test, cardiovascular responses were defined as the difference between the median value in the last 3 seconds of the handgrip procedure and the median value of the last 30 seconds before initiation of the exercise. The short epoch (3 seconds length) during handgrip was chosen in order to obtain the most extreme (maximal) value in

each individual (Nygaard et al. 2019). Likewise, for the Valsalva maneuver, HR response was defined as the largest positive (tachycardia) and negative (bradycardia) change from baseline (ie., median value of the last 30 seconds before initiation of the maneuver) (Christensen et al. 2020). SBP fall was taken as the largest negative change from baseline during the maneuver, whereas SBP overshoot was defined as the largest positive difference between baseline and the recovery phase immediately after the maneuver. In addition, for the Valsalva maneuver, recordings from each experimental run at inclusion and 12 months were converted to a 4 Hz time series of equal length by linear interpolation using Mathcad (PTC Mathcad 15.0, PTC, Needham, Massachusetts, USA). The resulting time series were normalized, taking the mean value of the baseline period as zero, and coherent averaging was performed by calculating the arithmetical mean for each time point (Rompelman & Ros 1986).

Other Variables

Background information was obtained from the medical records. Right heart catheterization with pressure recordings (including mean right atrial pressure (RAP)) based on standard electronic transducers as well as routine surveillance myocardial biopsies were performed 3 weeks, 6 months and 12 months after HTx. Treadmill testing was performed at inclusion and 12 months (Nytrøen et al. 2019). Also, comprehensive echocardiographic assessments were performed at inclusion and 12 months. Cardiac ejection fraction was obtained using the Simpson biplane method.

Statistics

Statistical analyses were carried out using SPSS statistical software (SPSS Inc., Chicago Illinois, USA). Results are presented with mean (standard deviation) or median (interquartile range) and corresponding confidence intervals (CIs), depending on distribution. As the

number of missing values was very limited, imputation was not considered necessary. Changes in variables in the HTxR group over time (from baseline to 6 months and from baseline to 12 months, respectively) were assessed using the paired sample t-test or the Wilcoxon test, as appropriate. The relationship between mean RAP obtained from cardiac catheterization and HR responses during 20º HUT was explored by linear regression analyses. A p-value ≤ 0.05 was considered statistically significant, and all tests were carried out two-sided. As several variables are strongly intercorrelated, p-values were not adjusted for test multiplicity.

Results

A total of 50 HTxR were included at baseline (35 males and 15 females, mean age 48 years). Of those, 45 (90%) were successfully followed-up at 6 months, and 47 (94%) at 12 months (Figure 1, Table 1). In addition, a group of 50 healthy controls was examined once. VO_2 and estimated glomerular filtration rate (GFR) increased during the follow-up period, whereas NT-pBNP and mean RAP decreased in the HTxR group. The usage of diuretics decreased during the follow-up period, however, there were hardly any other changes in cardiovascular active drugs (Table 1).

At supine rest, LF-RRI increased significantly from baseline to 6 months, and there was also a small but significant increase in HR (Table 2). LF-RRI indices further increased to 12 months. LF-SBP increased gradually throughout the follow-up period, reaching statistical significance at 12 months, as compared with baseline. Blood pressures and end-diastolic volume index (EDVI) did not change, and remained higher and lower, respectively, in the HTxR group as compared to healthy controls.

In response to 20º HUT, the HTxRs developed a HR response over the 12 months follow-up period that approached the one seen in healthy controls (Table 3). The EDVI response became more negative, but remained far from normal. There was a tendency towards enhanced BP and TPRI responses in the HTxR group, but not to the level of statistical significance. The association between resting mean RAP and HR response during 20º HUT was non-significant at baseline and 6 months, but became strongly negative at 12 months in the HTxR group (Table 4).

During the Valsalva maneuver, a similar EDVI decline in the HTxR group at baseline and 12 months was accompanied by a stronger TPRI increase at 12 months (Figure 2). Correspondingly, the SBP overshoot among HTxRs increased significantly over the

observation period (Table 5). During isometric exercise, EDVI and TPRI responses were similar at all time points in the HTxR group (Table 6).

At 12 months follow-up, HTxRs who were off all cardiovascular drugs had higher resting EDVI and a stronger HR response to 20^{o} HUT as compared the subgroup that were maintained on cardiovascular medication (Supplementary Table 1). Other variables did not differ importantly between the two subgroups.

Discussion

Taken together, the results of the functional assessments in the present study strongly suggest the occurrence of afferent reinnervation of low-pressure cardiopulmonary receptors during the first year after HTx. This is a novel finding.

The 20° HUT procedure primarily unloads low-pressure cardiopulmonary receptors, which in turn leads to a reflex increase in sympathetic efferent activity to both the vasculature and the heart (Triedman et al. 1993, Wyller et al. 2007). The gradual normalization of the HR response to light orthostatic challenge during the follow-up period directly suggests that both the afferent cardiopulmonary and efferent sympathetic limbs of this reflex loop are intact. The efferent sympathetic reinnervation of the sinus node demonstrated here has been reported elsewhere (Christensen et al. 2020); however, the afferent reinnervation of cardiopulmonary receptors that re-establishes a normal reflex loop is a novel finding of the present study. Likewise, the strong negative relationship between resting mean RAP and the HR response to 20° HUT at 12 months, which was not observed at baseline nor 6 months, indicates that high filling pressure has an inhibitory effect on sympathetically mediated HR control, which in turn requires cardiopulmonary receptor reinnervation and a restored reflex loop. Normally, 20° HUT also results in a significant TPRI response, as can be seen in the healthy control group (Triedman et al. 1993, Wyller et al. 2007). Accordingly, the TPRI responses approached normality during the follow-up period in the HTxR group, albeit not to the degree of statistical significance.

The spontaneous fluctuations of EDVI is an important source of blood pressure variability in the low-frequency band in healthy individuals. The causal relationship involves stimulation of low-pressure cardiopulmonary receptors and a reflex-mediated response of arterial resistance vessels, which in turn alters TPRI and thereby blood pressure (Mukkamala et al. 2005; Aletti et al. 2012; Aletti et al. 2013). During transplant surgery, the afferent part of

the reflex loop from cardiac cardiopulmonary receptors is destroyed; however, the remaining elements of the causal chain remain intact. Thus, the significant increase of resting LF-SBP power during the first year after HTx indicates cardiac cardiopulmonary receptor reinnervation. Of note, LF-SBP power is also strongly dependent on the arterial baroreceptor reflex control of peripheral resistance vessels; a complete elimination of LF-SBP power at baseline is therefore not expected, in line with our observations.

Normally, tonic stimulation of cardiopulmonary receptors reflexively reduces sympathetic outflow to the peripheral vasculature at rest (Triedman et al. 1993; Goldstein 2001); thus, a lack of afferent impulses would presumably enhance sympathetic vasoconstriction of arterial resistance vessels as well as venous capacitance vessels. The latter may explain why the EDVI response to 20^{o} HUT was abolished in the HTxR group at baseline (the confidence interval includes zero). However, the small but statistically significant increase in EDVI responsiveness (the value becoming more negative over the follow-up period), suggests an increase of venous capacitance, which in turn might indicate a slight decrease in sympathetic vasomotion due to cardiopulmonary receptor reinnervation. Accordingly, we observed a slight (but not statistically significant) reduction of resting TPRI in the HTxR group over the follow-up period.

We also suggest that cardiopulmonary reinnervation could explain the somewhat puzzling observations regarding HR control at rest. At 6 months follow-up, there was a slight but significant increase in resting HR as compared with baseline among the HTxRs, an observation that could be interpreted as an early sign of efferent sympathetic reinnervation (Christensen et al. 2020). The apparent reversal of this observation at 12 months – while other indices of sympathetic reinnervation were normalized further – might be the effect of reflexive inhibition of sinus node sympathetic activity at rest due to partially restored afferent connections from cardiopulmonary receptors.

The Valsalva maneuver affects both arterial and cardiopulmonary baroreceptors (Hamilton et al. 1936, Goldstein et al. 1982). However, the MAP as well as the EDVI reduction during phase 2 of the maneuver was closely similar at baseline and 12 months in the HTxR group. If the arterial baroreceptor control of TPRI is unaffected by heart transplantation, any differences in TPRI responses between the two time points should be related to different cardiopulmonary receptor reflexes. Thus, the observed stronger increase in TPRI during Valsalva at 12 months, causing a strong blood pressure overshoot in phase 4, is coherent with cardiopulmonary receptor reinnervation.

As opposed to the Valsalva maneuver, autonomic adjustments during isometric exercise primarily depend on central command, whereas baroreceptor reflexes play a minor role (Goodwin et al. 1972, Kamiya et al. 2000). Accordingly, the TPRI responses did not change in the HTxR group over the follow-up period, suggesting that the observed differences in TPRI responsiveness during HUT and Valsalva are specific to these experiments, and not a feature of generalized altered peripheral vessel dynamics.

Taken together, the results from the functional assessments in the present study suggest that a high filling pressure might reduce sympathetic outflow to the heart and peripheral resistance vessels through partly restored cardiopulmonary receptor reflex mechanism. Thus, afferent reinnervation of cardiopulmonary receptors might have a counter-acting rather than contributing role towards hypertension development late after HTx. Furthermore, this appears to be an argument against the use of diuretics for hypertension treatment in HTxRs. This question should be scrutinized in a clinical trial.

Generally, afferent reinnervation after HTx has not been well addressed in previous studies (Stark et al. 1991). The present results add to the limited knowledge of afferent reinnervation, and suggests stronger focus on this topic in future research.

Strengths and Limitations

Cardiopulmonary receptors are not confined to the heart, but are also located in other parts of the cardiopulmonary circulation, such as the main pulmonary artery (Goldstein 2001). These receptors and their afferent neural connections might be unaffected by heart transplantation surgery. Thus, an alternative explanation for the results reported in the present study is sensitization of reflex loops that emanates from these non-cardiac receptors. While such a phenomenon certainly may confound a conclusion of afferent reinnervation, the physiological and clinical consequences might still be comparable.

Data on DBP variability might have provided additional information cardiopulmonary baroreflex function (Aletti et al. 2012). Unfortunately, such data were not available for the present study due to technical limitations. Likewise, continuous measurements of RAP as well as venous pressures during the autonomic tests might have yielded better insight into the reflex mechanisms, including more subtle physiological phenomena such as the Bainbridge reflex (Barbieri et al. 2002).

During the 12 months follow-up period, some of the individuals in the HTxR group had alterations in the use of cardiovascular pharmaceuticals. This introduces a possible bias; however, it should be noted that the subgroups off and on cardiovascular drugs at 12 months, respectively, were closely similar for most relevant variables. Unfortunately, we do not have information on the underlying rationale for changes in pharmaceutical regimens.

A strength of the present study is the prospective design with high patient compliance, combined with thorough autonomic cardiovascular assessment.

Conclusion

Functional assessment suggests that afferent reinnervation of low-pressure cardiopulmonary receptors occurs during the first year after heart transplantation, partly restoring reflexively mediated responses to altered cardiac filling. This is a novel finding; its consequences should be addressed in further research.

Declarations

Author contributions

SN and AHC collected clinical data, contributed to study design and participated in data analyses. KR, KN, LG, AF, ET, GD and JPS supervised data analyses and contributed to study design. VBBW conceived of the study, contributed to study design and participated in data analyses. JPS is funded by the IDeA States Pediatric Clinical Trials Network (ISPCTN) of the National Institutes of Health, Grant #UG1OD024949. All authors contributed to data interpretation and drafting of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Compliance with ethical standards

- Conflicts of interest: The authors declare that they have no conflicts of interest.
- Ethical approval: All procedures performed in the present study were in accordance with the ethical standards of the Norwegian National Committee for Ethics in Medical research and with the 1964 Helsinki declaration and its later amendments. This article does not contain any studies with animals performed by any of the authors.
- Informed consent: Informed consent was obtained from all individual participants included in the study.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1. Study populations

	HTx baseline (n=50)	*HTx 6 months (n=45)*	*HTx 12 months (n=47)*	*Healthy controls (n=50)*
Recipient characteristics				
Sex - no. (%)				
Male	35 (70)	33 (73)	33 (70)	35 (70)
Female	15 (30)	12 (27)	14 (30)	15 (30)
Age, years - mean (SD)	48,2 (13,0)	n.a.	n.a.	47,8 (12,4)
BMI, kg/m^2 - mean (SD) 95% CI	24,8 (3,8) 23,7 to 25,9	25,5 (3,9) 24,4 to 26,7	26,4 (4,3) 25,1 to 27,7	25,2 (3,0) 24,3 to 26,1
Cardiovascular pharmaceuticals				
Adrenergic beta-blocker - no. (%)	14 (28)	14 (31)	11 (23)	n.a.
Calcium channel blocker - no. (%)	13 (26)	12 (27)	12 (26)	n.a.
ACE/AT-II blocker - no. (%)	3 (6)	4 (9)	4 (9)	n.a.
Diuretics - no. (%)	40 (80)	32 (71)	20 (43)	n.a.
Immunosupression				
Ciclosporin - no. (%)	36 (72)	31 (69)	27 (57)	n.a.
Tacrolimus - no. (%)	14 (28)	11 (24)	12 (26)	n.a.
Everolimus - no. (%)	14 (28)	17 (38)	18 (38)	n.a.
Prednisolone - no. (%)	50 (100)	45 (100)	47 (100)	n.a.
Mycophenolate - no. (%)	47 (94)	38 (84)	41 (87)	n.a.
Biochemistry				
HbA1c, % - mean (SD) 95% CI	5,68 (0,74) 5,47 to 5,89	5,91 (0,77) 5,68 to 6,14	5,72 (0,64) 5,53 to 5,90	5,24 (0,32) 5,15 to 5,33
Estimated GFR, ml/min/1.73 m^2 - mean (SD) 95% CI	56 (17) 51 to 61	57 (15) 53 to 62	70 63 to 76	92 (19) 87 to 98
NT-pBNP, ng/L - median (IQR) 95% CI	901 (926) 634 to 1260	304 (359) 254 to 457	254 (355) 203 to 389	51 (66) 28 to 71
Functional asssessments				

Biplane ejection fraction, % - mean (SD) 95% CI	58 (5,4) 57 to 60	n.a.	58 (5,7) 56 to 59	58 (4,5) 57 to 59
Mean right atrium pressure (RAP), mmHg - mean (SD) 95% CI	4,6 (3,7) 3,7 to 5,7	3,8 (2,9) 2,9 to 4,7	3,5 (2,7) 2,7 to 4,3	n.a.
VO_2-peak, ml/kg/min - mean (SD) 95% CI	21,1 (5,0) 19,7 to 22,6	n.a.	25,3 (7,2) 23,2 to 27,5	40,3 (7,9) 38,0 to 42,5
Rejections				
Cellular Rejections - no. (%)				
No rejection	32 (64)	43 (96)	44 (94)	n.a.
R1	10 (20)	2 (4)	3 (6)	n.a.
R2	7 (14)	0 (0)	0 (0)	n.a.
Humoral rejections - no. (%)	1 (2)	0 (0)	0 (0)	n.a.

SD=standard deviation; IQR=interquartile range; CI=confidence interval; HTx=heart transplantation; ACE=angiotensine converting enzyme; AT-II=angotensine receptor II; GFR=glomerulus filtration rate; BNP=brain natriuretic protein; SBP=systolic blood pressure; DBP=diastolic blood pressure; n.a.=not applicable. Troponin T < 5 from the lab records is reported as 5; estimated GFR > 60 from the lab records is reported as 60. Rejections are summarized from the intervals HTx to inclusion, inclusion to 6 months, 6 months to 12 months.

Table 2. Development of resting haemodynamic and autonomic variables in HTxR over time. Healthy controls for comparison

	HTx baseline	*HTx 6 months*	*p-value**	*HTx 12 months*	*p-value**	*HC*
HR, beats/min - mean (SD) 95% CI	82 (10) 79 to 85	84 (12) 80 to 87	**0,050**	83 (10) 80 to 86	0,271	56 (8) 54 to 58
SBP, mmHg - mean (SD) 95% CI	118 (14) 114 to 122	117 (14) 112 to 121	0,454	117 (15) 113 to 122	0,936	109 (13) 105 to 113
DBP, mmHg - mean (SD) 95% CI	79 (10) 76 to 82	80 (12) 76 to 83	0,621	79 (9) 76 to 82	0,537	72 (10) 69 to 75
MAP, mmHg - mean (SD) 95% CI	90 (10) 87 to 93	90 (12) 87 to 94	0.977	90 (10) 87 to 93	0.614	83 (11) 80 to 86
CI, l/min/m^2 - mean (SD) 95% CI	2.4 (0.4) 2.3 to 2.5	2.4 (0.5) 2.3 to 2.5	0,633	2.4 (0.5) 2.2 to 2.5	0,483	2.4 (0.6) 2.2 to 2.5

TPRI, mmHg/l/min/m^2 - mean (SD) 95% CI	10.8 (2.9) 9.9 to 11.6	10.3 (2.9) 9.4 to 11.2	0,300	10.3 (3.0) 9.4 to 11.3	0,427	9.2 (2.5) 8.5 to 9.9
EDVI, ml/m^2 - mean (SD) 95% CI	51.5 (8.7) 49.0 to 54.0	50.9 (8.8) 48.2 to 53.5	0,328	50.9 (9.9) 47.9 to 53.8	0,163	68.6 (12.6) 65.0 to 72.1
LF_RRI, ms^2 - median (IQR) 95% CI	0,49 (2,5) 0,33 to 1,1	1,4 (5,7) 0,54 to 2,6	**0,012**	2,5 (6,0) 1,5 to 4,7	**<0,001**	511 (806) 326 to 720
LF_RRI, nu - mean (SD) 95% CI	19.9 (14.8) 15.7 to 24.1	29.6 (18.0) 24.2 to 35.0	**0,014**	36.6 (24.3) 29.4 to 43.8	**<0,001**	58.1 (19.8) 52.5 to 63.7
HF_RRI, ms^2 - median (IQR) 95% CI	4.0 (12.0) 1.9 to 9.9	4.0 (10.6) 1.7 to 7.3	0,474	3.3 (7.6) 2.7 to 5.6	0,185	284 (745) 221 to 422
HF_RRI, nu - mean (SD) 95% CI	80 (15) 76 to 84	70 (18) 65 to 76	**0,014**	63 (24) 56 to 71	**<0,001**	42 (20) 36 to 48
LF/HF_RRI - median (IQR) 95% CI	0.23 (0.30) 0.14 to 0.28	0.35 (0.51) 0.24 to 0.47	**0,006**	0.48 (0.93) 0.30 to 0.71	**<0,001**	1.8 (2.5) 1.2 to 2.2
Total power_RRI, ms^2 - median (IQR) 95% CI	5.1 (16.6) 3.1 to 10.7	6.5 (21) 3.4 to 13.5	0,144	10.7 (30.5) 7.1 to 19.2	**0,001**	1368 (1751) 942 to 2045
LF_SBP, mmHg^2 - median (IQR) 95% CI	2.9 (5.7) 1.7 to 4.2	3.8 (8.2) 2.4 to 7.3	0,056	5.3 (13.4) 3.7 to 13.3	**0,013**	7.0 (9.7) 4.6 to 9.5
LF_SBP, nu - mean (SD) 95% CI	33 (13) 29 to 36	35 (15) 30 to 40	0,242	39 (13) 35 to 44	**0,006**	35 (13) 31 to 39
HF_SBP, mmHg^2 - median (IQR) 95% CI	1.3 (3.1) 1.1 to 1.9	2.3 (3.9) 1.4 to 3.3	0,156	2.3 (3.3) 1.6 to 3.7	0,457	2.4 (3.1) 1.8 to 3.7
HF_SBP, nu - mean (SD) 95% CI	20 (12) 17 to 24	19 (15) 15 to 24	0,738	15 (10) 12 to 19	**0,003**	17 (13) 13 to 21
Total power_SBP, mmHg^2 - median (IQR) 95% CI	8.2 (17.4) 5.2 to 15.4)	12.9 (23.6) 9.2 to 23.4	0,057	15.5 (28.6) 11.5 to 29.0)	**0,036**	20.2 (24.5) 11.0 to 26.8

*p-values are computed applying paired samples t-tests (6 months values compared with baseline and 12 months values compared with baseline, respectively). Variables deviating strongly from a normal distribution were ln-transformed prior to testing. P-values <0,05 are highlighted for clarity. A Bonferroni-adjustment suggest a level of signifiance at 0.05/36=0.001. HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial blood pressure; CI=cardiac index; TPRI=total peripheral resistance index; EDVI=end diastolic volum index; LF_RRI=Low-frequency power of RR-interval variability; HF_RRI=High-frequency power of RR-interval variability; LF_SBP=low-frequency power of systolic blood pressure variability; HF_SBP=high-frequency power of systolic blood pressure variability; SD=standard deviation; CI=confidence interval; IQR=interquartile range.

Table 3. Development of responses to 20 deg. head-up tilt in HTxR over time. Healthy controls for comparison

	HTx baseline	*HTx 6 months*	*p-value**	*HTx 12 months*	*p-value**	*HC*
Δ HR, beats/min - mean (SD) 95% CI	-0.6 (1.5) -1.0 to -0.2	0.9 (2.8) 0.1 to 1.8	**0,002**	1.9 (3.1) 1.0 to 2.8	**<0.001**	2.2 (2.6) 1.5 to 3.0
Δ SBP, mmHg - mean (SD) 95% CI	0.9 (10.6) -2.3 to 4.1	4.2 (11.8) 0.5 to 8.0	0,365	3.5 (7.1) 1.2 to 5.8	0,236	7.1 (6.8) 5.0 to 9.1
Δ DBP, mmHg - mean (SD) 95% CI	3.7 (8.1) 1.3 to 6.1	5.8 (7.6) 3.3 to 8.2	0,684	6.1 (5.2) 4.4 to 7.7	0,220	9.1 (4.5) 7.8 to 10.5
Δ MAP, mmHg - mean (SD) 95% CI	2.5 (9.0) -0.1 to 5.2	5.6 (9.1) 2.7 to 8.5	0.375	5.2 (5.7) 3.4 to 7.1	0.161	8.5 (5.4) 6.9 to 10.1
Δ CI, l/min/m^2 - mean (SD) 95% CI	-0.04 (0.19) -0.1 to 0.01	-0.08 (0.15) -0.13 to -0.04	0,386	-0.08 (0.18) -0.14 to -0.03	0,297	-0.27 (0.31) -0.36 to -0.18
Δ TPRI, mmHg/l/min/m^2 - mean (SD) 95% CI	0.4 (1.5) -0.1 to 0.8	1.0 (1.4) 0.6 to 1.5	0,131	1.0 (1.1) 0.6 to 1.3	0,094	2.0 (1.4) 1.6 to 2.4
Δ EDVI, ml/m^2 - mean (SD) 95% CI	1,0 (4,4) -0.2 to 2.2	-1.0 (2.9) -1.9 to -0.1	**0,012**	-1.0 (3.4) -2.0 to -0.01	**0,006**	-7.8 (7.3) -9.9 to -5.7
Δ LF_RRI, ms^2 - median (IQR) 95% CI	-0.2 (0.9) -0.32 to -0.09	-0.1 (1.7) -0.51 to 0.07	0.477	-0.03 (1.6) -0.33 to 0.07	0.924	-84 (328) -147 to -22
Δ LF_RRI, nu - mean (SD) 95% CI	-2.5 (8.5) -4.9 to 0.01	-0.4 (12.9) -4.3 to 3.5	0,579	0.4 (12.0) -3.4 to 4.0	0,102	0.8 (11.8) -2.6 to 4.2
Δ HF_RRI, ms^2 - median (IQR) 95% CI	-0.14 (3.7) -1.2 to 0.4	-0.15 (5.6) -1.4 to 0.5	0,726	0.05 (5.2) -0.5 to 0.7	0,335	-95 (266) -188 to -41
Δ HF_RRI, nu - mean (SD) 95% CI	2.5 (8.5) 0.01 to 4.9	0.4 (12.9) -3.5 to 4.3	0,579	-0.4 (12.0) -4.0 to 3.1	0,102	-0.8 (11.8) -4.2 to 2.6
Δ LF/HF_RRI - median (IQR) 95% CI	-0.03 (0.10) -0.04 to 0.0	-0.02 (0.24) -0.06 to 0.04	0,916	-0.003 (0.28) -0.07 to 0.04	0,561	0.09 (0.78) -0.1 to 0.3
Δ Total power_RRI, ms^2 - median (IQR) 95% CI	-0.3 (6.0) -1.9 to 0.3	-0.8 (8.7) -3.0 to 1.1	0,657	-0.01 (9.8) -2.0 to 1.5	0,276	-276 (605) -418 to -122

Δ LF_SBP, $mmHg^2$ - median (IQR) 95% CI	-0.3 (1.2) -0.63 to -0.11	-0.3 (1.2) -0.65 to -0.06	0,950	-0.6 (1.2) -1.1 to -0.21	0,287	-0.6 (1.5) -1-0 to -0.21
Δ LF_SBP, nu - mean (SD) 95% CI	2.3 (7.9) 0.01 to 4.7	2.8 (7.0) 0.5 to 5.0	0,931	1.9 (7.6) -0.5 to 4.4	0,857	0.7 (5.9) -1.0 to 2.4
Δ HF_SBP, $mmHg^2$ - median (IQR) 95% CI	-0.1 (0.2) -0.1 to 0.01	-0.1 (0.4) -0.3 to -0.07	**0,004**	-0.2 (0.4) -0.47 to -0.14	**0,001**	-0.2 (0.5) -0.3 to -0.06
Δ HF_SBP, nu - mean (SD) 95% CI	1.5 (3.4) 0.5 to 2.5	0.7 (2.9) -0.2 to 1.6	0,233	0.1 (2.6) -0.7 to 0.9	0,120	0.6 (2.0) -0.04 to 1.2
Δ Total power_SBP, $mmHg^2$ - median (IQR) 95% CI	-1.5 (4.0) -2.9 to -0.9	-1.6 (3.1) -2.3 to -1.1	0,451	-2.2 (3.9) -3.5 to -1.4	0,179	-1.7 (5.3) -3.0 to -0.6

*p-values are computed applying paired samples t-tests or Wilcoxon test as appropriate (6 months values compared with baseline and 12 months values compared with baseline, respectively). P-values <0,05 are highlighted for clarity. A Bonferroni-adjustment suggest a level of signifiance at 0.05/36=0.001. HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial blood pressure; CI=cardiac index; TPRI=total peripheral resistance index; EDVI=end diastolic volum index; LF_RRI=Low-frequency power of RR-interval variability; HF_RRI=High-frequency power of RR-interval variability; LF_SBP=low-frequency power of systolic blood pressure variability; HF_SBP=high-frequency power of systolic blood pressure variability; SD=standard deviation; CI=confidence interval; IQR=interquartile range.

Table 4. Relationship between mean right atrial pressure (obtained from cardiac catheterization) and heart rate responses during 20 deg. HUT (cf. Table 3). Bivariate linear regression models

	HTx baseline	*p-value**	*HTx 6 months*	*p-value**	*HTx 12 months*	*p-value**
Mean RAP → Δ HR, reg. coeff. B 95% CI	-0,02 -0.14 to 0.10	0,697	0,06 -0.24 to 0.35	0,704	-0,58 -0.88 to -0.27	**<0.001**

*p-values are from the within-group linear regression analyses. HR=heart rate; RAP=right atrial pressure; CI=confidence intervals; HUT=head-up tilt-test

Table 5. Development of responses to Valsalva maneuver in HTxR over time. Healthy controls for comparision

	HTx baseline	*HTx 6 months*	*p-value**	*HTx 12 months*	*p-value**	*HC*
Tachycardia response, beats/min - mean (SD) 95% CI	1.8 (1.1) 1.4 to 2.1	3.7 (2.5) 2.9 to 4.4	**<0,001**	6.4 (4.6) 5.0 to 7.7	**<0,001**	15.3 (7.5) 13.1 to 17.5
Bradycardia response, beats/min - mean (SD) 95% CI	-0.9 (1.6) -1.4 to -0.4	-0.7 (1.3) -1.1 to -0.3	0,326	-1.0 (1.4) -1.4 to -0.6	0,899	-6.2 (3.9) -7.4 to -5.1
SBP fall, mmHg - mean (SD) 95% CI	-25 (14) -29 to -20	-22 (11) -25 to -18	0,396	-23 (11) -26 to -19	0,125	-19 (9) -22 to -17
SBP overshoot, mmHg - mean (SD) 95% CI	8.1 (7.7) 5.7 to 10.4	11.8 (8.9) 9.0 to 14.7	**0,014**	14.0 (8.9) 11.2 to 16.9	**0,026**	9.7 (10.3) 6.7 to 12.7

*p-values are computed applying paired samples t-tests (6 months values compared with baseline and 12 months values compared with baseline, respectively). A Bonferroni-adjustment suggest a level of signifiance at 0.05/8=0.01. SBP=systolic blood pressure; SD=standard deviation; CI=confidence interval

Table 6. Development of responses to isometric exercise (handgrip) in HTxR over time. Healthy controls for comparison

	HTx baseline	*HTx 6 months*	*p-value**	*HTx 12 months*	*p-value**	*HC*
Δ HR, beats/min - mean (SD) 95% CI	-0.3 (0.7) -0.5 to -0.1	0.7 (1.5) 0.3 to 1.2	**<0,001**	2.3 (2.9) 1.4 to 3.2	**<0,001**	5.4 (5.3) 3.9 to 7.0
Δ SBP, mmHg - mean (SD) 95% CI	15 (9) 12 to 18	18 (10) 15 to 22	**0,057**	21 (12) 17 to 25	**0,018**	17 (8) 15 to 19
Δ TPRI, mmHg/l/min/m^2 - mean (SD) 95% CI	1.5 (0.9) 1.2 to 1.8	1.7 (1.1) 1.3 to 2.0	0,191	1.8 (1.2) 1.4 to 2.1	0,224	1.3 (1.2) 1.0 to 1.7
Δ EDVI, ml/m^2 - mean (SD) 95% CI	0.02 (2.0) -0.6 to 0.6	0.2 (3.3) -0.8 to 1.2	0,941	-0.1 (6.1) -2.1 to 1.9	0,713	-2.5 (4.9) -3.9 to -1.1

*p-values are computed applying paired samples t-tests (6 months values compared with baseline and 12 months values compared with baseline, respectively). A Bonferroni-adjustment suggest a level of signifiance at 0.05/12=0.004. HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; CI=cardiac index; TPRI=total peripheral resistance index; EDVI=end diastolic volum index; LF_RRI=Low-frequency power of RR-interval variability; LF_SBP=low-frequency power of systolic blood pressure variability; SD=standard deviation; CI=confidence interval

Figure legends

Figure 1

Study flowchart.

Figure 2

Graphical depiction of cardiovascular responses to the Valsalva-maneuver at inclusion (red) and 12 months (green) among HTxRs; healthy controls (blue) are shown for comparisons. The graphs are constructed from coherent averaging of individual responses, cf. manuscript text for further details. The four phases of the Valsalva-maneuver are indicated with vertical lines. EDVI=end diastolic volume index; CI=cardiac index; mBP=mean blood pressure; TPRI=total peripheral resistance index; HR=heart rate.