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Heart rate variability and baroreflex sensitivity in bilateral lung transplant recipients

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Summary

The effects of lung afferents denervation on cardiovascular regulation can be assessed on bilateral lung transplantation patients. The high-frequency component of heart rate variability is known to be synchronous with breathing frequency. Then, if heart beat is neurally modulated by breathing frequency, we may expect disappearance of high frequency of heart rate variability in bilateral lung transplantation patients. On 11 patients and 11 matching healthy controls, we measured R-R interval (electrocardiography), blood pressure (Portapres®) and breathing frequency (ultrasonic device) in supine rest, during 10-min free breathing, 10-min cadenced breathing (0·25 Hz) and 5-min handgrip. We analysed heart rate variability and spontaneous variability of arterial blood pressure, by power spectral analysis, and baroreflex sensitivity, by the sequence method. Concerning heart rate variability, with respect to controls, transplant recipients had lower total power and lower low- and high-frequency power. The low-frequency/high-frequency ratio was higher. Concerning systolic, diastolic and mean arterial pressure variability, transplant recipients had lower total power (only for cadenced breathing), low frequency and low-frequency/high-frequency ratio during free and cadenced breathing. Baroreflex sensitivity was decreased. Denervated lungs induced strong heart rate variability reduction. The higher low-frequency/high-frequency ratio suggested that the total power drop was mostly due to high frequency. These results support the hypothesis that neural modulation from lung afferents contributes to the high frequency of heart rate variability.

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arterial blood pressure; autonomic nervous system; cardiovascular physiology; lung denervation; transplantation

Introduction

Bilateral lung transplantation (BLT) carries along denervation of lung afferent cardiovascular sensitive nerves so that the graft is no longer connected to the autonomic nervous system (ANS) (Schaefers *et al.*, 1990). Thus, BLT is an excellent experimental model for the study of cardiovascular regulation without modulation of heart activity by parasympathetic and/or sympathetic lung afferents. Breathing frequency (BF) is synchronous with and modulates the high-frequency peak (HF) of spontaneous heart rate variability (HRV) (Malliani *et al.*, 1991; Taha *et al.*, 1995; Perini & Veicsteinas, 2003). If the ANS is the main modulator of HRV, then the HF of HRV would have to disappear after BLT.

The studies of autonomic modulation of HRV in BLT recipients (BLTR) are scanty (Schaefers *et al.*, 1990; Iber *et al.*, 1995). Some were on children (Dalla Pozza *et al.*, 2006; Pozza *et al.*, 2006) and pointed to a demonstration of possible lung reinnervation after transplantation. Single lung transplant recipients were compared with either BLTR (Berakis *et al.*, 2002) or lung and heart–lung transplant recipients (Seals *et al.*, 1993). No clear conclusion emerged on this subject from those works.

The aim of this study was to analyse the effects of lung denervation on HRV in BLTR as compared with healthy controls. The hypothesis was that the modulation of the HF component of HRV by the breathing frequency is mediated by the ANS. This hypothesis would be supported by the results if HF of HRV is suppressed in BLTR.

Experimental methods

Subjects

Eleven BLTR (Six women and five men) participated in the experiments. They were all clinically stable, clinical stability being defined by the absence of concomitant infectious or

Table 1 Antirejection medication in every bilateral lung transplant recipient.

Patients	Medication	
1	Everolimus	Prednisolone
2	Tacrolimus	Prednisolone
3	Tacrolimus	Prednisolone
4	Tacrolimus	Prednisolone
5	Tacrolimus	Prednisolone
6	Tacrolimus	Mycophénolate mofétil Prednisolone
7	Tacrolimus	Azathioprine
8	Everolimus	Prednisolone
9	Tacrolimus	Prednisolone
10	Tacrolimus	Prednisolone
11	Tacrolimus	Prednisolone

inflammatory disease, stable pulmonary function tests and without evidence of chronic or acute rejection or any other disease affecting the ANS. Patients with diabetes, either induced by pancreatic manifestations of cystic fibrosis or by immunosuppressant treatment, were excluded from the study. The patients' age ranged from 26 to 68 (mean value 49·0 ± 14·5 years). They were 168 ± 10 cm tall and weighed 62·6 ± 12·0 kg. Their transplant surgery took place between 6 months and 12 years (mean 3·9 ± 3·5 years) before their inclusion in the study. All their antirejection medications are reported in Table 1.

Eleven healthy non-smoking subjects were recruited as controls so that each BLTR was coupled with a matching control for gender, age (±2 years), height (±5 cm) and weight (±5 kg). Their age ranged from 25 to 69 (47·8 ± 14·1). They were 168 ± 10 cm tall and weighed 63·6 ± 8·9 kg. They had no history of cardiopulmonary disease and were not taking medications at the time of the experiments.

The subjects, all non-smokers since at least the surgery, abstained from coffee-containing products during the 12 h before the experiment. Experiments were carried out in the morning, between 9 and 11 a.m. On the day of testing, they had a light breakfast at 7 a.m. They were preliminarily informed of aims, design, protocol, procedures and risks associated with the experiments. Informed consent was obtained from each volunteer, who was aware of the right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional ethical committee.

Measurements

The R-R interval (RR) was continuously measured by electrocardiography (Elmed ETM 2 000, Heiligenhaus, Germany). Continuous recordings of arterial pulse pressure were obtained at the middle phalanx of the left hand's second finger, using a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, The Netherlands). Systolic and diastolic blood pressure (SAP and DAP, respectively) were computed from each pulse pressure profile, using the Beatscope® software package (FMS, Amsterdam, The Netherlands). Beat-to-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the same software package. The BF was measured by an ultrasound device (Spiroson, Ecomedics, Suisse) plugged on a respiratory mask.

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 400 Hz.

Protocol

After instrumentation, the subject assumed the supine posture. After 5 min of quiet rest, the protocol started with 10 min of free breathing. It was followed by 10 min with cadenced breathing (0·25 Hz). Eventually, the subjects underwent 5 min of handgrip contractions (30–50 contractions per minute without controlled BF), to induce an increase in sympathetic activity. All investigated variables were continuously recorded during the entire protocol.

Data treatment

The time series of RR, from the continuous recordings of ECG, and that of SAP and DAP, from pulse pressure profiles, were constructed. On these time series, power spectral analysis was performed to evaluate spontaneous variability of RR, SAP and DAP (Pagani *et al.*, 1986). The total power (P_{tot}) of RR, SAP and DAP variabilities, corresponding to the signal variance in the 0·0–0·5 Hz frequency range, was initially obtained. Subsequently, the powers and frequencies of the low (LF, 0·04–0·15 Hz)- and the high (HF, 0·15–0·4 Hz)-frequency spectral components were computed and expressed in absolute units. The LF/HF ratio was also calculated. The very low-frequency (VLF) component was neglected, because it can be measured only during very long recordings and it is a source of noise in the present experimental circumstances (Furlan *et al.*, 1990).

Therefore, to infer on the relative weight of LF and HF in the spectra, they were normalized with respect to their sum made equal to 100% (LFnu et HFnu, respectively) after exclusion of the frequencies lower than 0·03 Hz (Malliani *et al.*, 1991; Pagani *et al.*, 1986; Task force, 1996). LFnu and HFnu were computed as:

$$\frac{LF \times 100}{P_{tot} - VLF} \text{ or } \frac{HF \times 100}{P_{tot} - VLF} \quad (1)$$

and expressed in normalized units (nu) (Malliani *et al.*, 1991)

At rest and exercise steady states, we also computed baroreflex sensitivity (BRS, expressed in ms $mmHg^{-1}$) by means of the sequence method (Bertinieri *et al.*, 1988). Briefly, sequences of three or more consecutive beats in which SAP and RR changed in the same direction, either increasing or

decreasing, were identified. A phase shift of one beat was introduced between the SAP and the RR values of each sequence, as in previous studies (Bertinieri *et al.*, 1988). Within each individual sequence, the RR versus SAP relationship was analysed by linear regression, to compute the slope and the corresponding coefficient of determination (r^2). Only slopes showing r^2 values higher than 0·85 were retained (Iellamo *et al.*, 1994, 1997). For each subject, the mean slope of the RR versus SAP relationships was then computed and taken as a measure of individual BRS, at rest and at exercise, respectively.

Spectral analysis and BRS were analysed by means of a purposely laboratory-made program written with MATLAB® software (Version 7·9·1 R2009b) (Costes *et al.*, 2004; Assoumou *et al.*, 2012; Dauphinot *et al.*, 2013; Fontolliet *et al.*, 2015).

Statistics

Data are reported as mean ± standard deviation (SD) for each experimental session. The effects of transplantation on the investigated parameters were analysed by two-way ANOVA (McDonald, 2014). When applicable, a Tukey *post hoc* test was used to locate significant differences. The Statistica 12© software package (StatSoft, Inc., Tulsa, OK, USA) was used. Differences were considered significant when $P<0·05$.

Results

Mean measured data

The mean values of measured and calculated cardiovascular variables in all investigated conditions are shown in Fig. 1. The heart rate was higher and thus RR lower, in BLTR than in controls, in all conditions. Cadenced breathing and handgrip did not affect RR. SAP, DAP and MAP were the same in patients and controls. With handgrip, SAP and DAP increased in control group and MAP increased in both groups. Baroreflex sensitivity was lower in BLTR than in controls, in every condition. Breathing frequency was higher in BLTR than in controls during free breathing (see Fig. 2).

Heart rate variability

The results of power spectral analysis for HRV are shown in Table 2, top panel, for all subjects. P_{tot} was lower in BLTR than in controls, because both the LF and the HF powers were lower. LFnu was higher and HFnu was lower in BLTR than in controls, in all investigated conditions. The LF/HF ratio was higher in BLTR than in controls in all conditions.

The absolute LF and HF powers of BLTR were characterized by large SD, because two patients were outliers: in fact, their LF and HF powers were so much higher than those of all other patients that they resulted more than two SDs above the mean group value (Fig. 3). These patients were thus extracted from the group and analysed separately in comparison with their respective control counterparts.

The bottom panel of Table 2 reports the results of power spectral analysis for HRV, for the remaining patients group ($n = 9$). All differences with respect to the control group were significant, as they were for $n = 11$, except for the LF power during handgrip. For $n = 9$, in BLTR compared to controls, P_{tot} was 93·3 ± 7·1% lower during free breathing, 89·7 ± 13·1% lower during cadenced breathing and 88·3 ± 8·9 lower during handgrip. The LF power was 82·7 ± 24·9% lower during free breathing and 94·3 ± 7·2% lower during cadenced breathing. The HF power was 95·1 ± 5·6% lower during free breathing, 97·6 ± 4·2% lower during cadenced breathing and 95·3 ± 3·2 lower during handgrip. Conversely, LFnu was higher and thus HFnu lower in BLTR than in controls, in all conditions. The LF/HF ratio was higher in the three investigated conditions.

Arterial pressure variability

The results of power spectral analysis for SAP, DAP and MAP are shown in Table 3. Concerning SAP, P_{tot} was lower in BLTR than in controls, in cadenced breathing (−51·0 ± 36·4%). The LF power was lower in BLTR than in controls, during free breathing (−41·8 ± 42·2%) and cadenced breathing (−72·6 ± 21·6%), but not during handgrip. HF power in patients was the same than in controls. The LF/HF ratio was lower in BLTR than in control, both in free breathing and in cadenced breathing.

Concerning DAP, P_{tot} and LF power were lower in patients than in controls in cadenced breathing (respectively, −42·2 ± 41·9% and −22·9 ± 84·7%) and also in free breathing for LF power (−37·2 ± 72·6%). HF power was not different between BLTR and controls in any conditions. The LF/HF ratio was lower in BLTR than in controls in free breathing (−50·2 ± 46·2%) and cadenced breathing (−9·6 ± 3·3%). Concerning MAP, P_{tot} and LF power were lower in patients than in controls in cadenced breathing (respectively, −23·2 ± 49·8% and −11·3 ± 114·4%) and also in free breathing for LF power (−20·1 ± 85·2%). The LF/HF ratio was lower in BLTR than in controls (−76·2 ± 77·2% in free breathing, −76·9 ± 66·4% in cadenced breathing and −50·7 ± 62·0% in handgrip).

The results of power spectral analysis for HRV of the two outliers and their respective control counterparts are shown in Table 4. All data were closer to controls' results than for the rest of the patients. Results were still lower for the two outliers than for their respective control counterparts.

Discussion

In this study, we tested the hypothesis that the modulation of the HF component of HRV by BF is mediated by afferent fibres from the lungs that are interrupted after BLT. To this aim, we investigated the effects of BLT on HRV. The tested

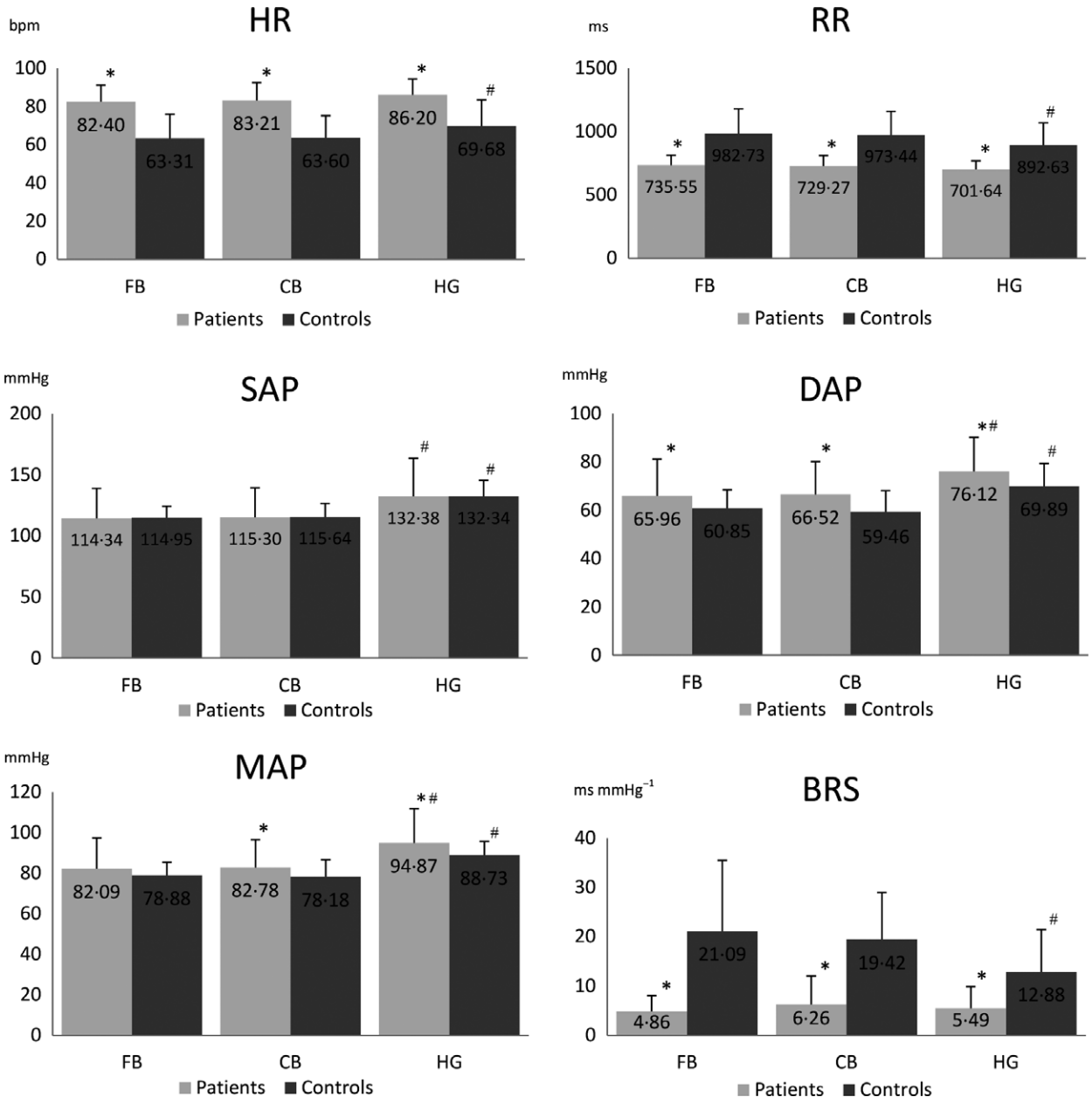

Figure 1 The mean cardiac values and standard deviations measured and calculated at steady state in supine position in all investigated conditions (n = 11). HR, heart rate; RR, R-R interval; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; BRS, baroreflex sensitivity; FB, free breathing; CB, cadenced breathing; HG, handgrip; *, significantly different from controls; #, significantly different from FB for the same group.

hypothesis would be fully supported by the results if the HF component of HRV was suppressed after BLT. The data show that P_{tot} and the LF and HF powers were strongly reduced in BLTR, a finding in substantial agreement with the tested hypothesis. We can, therefore, state that most of the spontaneous HR oscillations at rest are affected by neural afferents coming from the lungs. Nevertheless, some degree of spontaneous HRV remained in BLTR, suggesting that some of the overall HRV is related to other mechanisms than a modulation of cardiovascular centres by lung afferents. Moreover, if we look at normalized data, LFnu resulted higher and HFnu lower in BLTR than in controls, suggesting that the remaining variability in BLTR might have been under predominant sympathetic modulation.

We note, however, that in two BLTR spontaneous HRV was only partially reduced. Their LF and HF powers were more than two standard deviations above the mean group value. If the tested hypothesis was correct, this would mean that the neural efflux through lung afferents was present in these patients, implying the possibility of at least partial

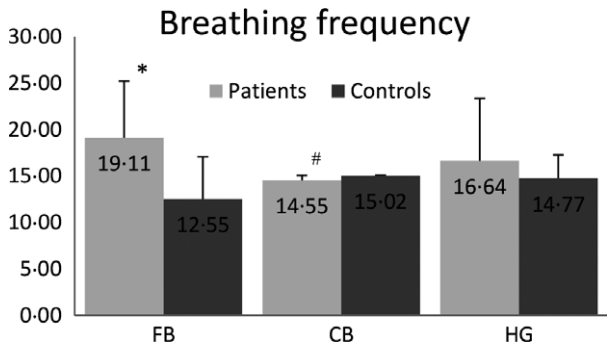

Figure 2 The mean breathing values and standard deviations measured at steady state in supine position in supine position in all investigated conditions (n = 11). FB, free breathing; CB, cadenced breathing; HG, handgrip; *, significantly different from controls; #, significantly different from FB for the same group.

reinnervation after complete lung denervation. This was not a direct effect of time after intervention, as the two patients who had, by far, the oldest surgery had very low HRV values, compared to other patients with more recent intervention. Antirejection medication cannot explain the different behaviour of the two outliers, as they had the same treatment as the other patients. In particular, they were both under tacrolimus, which if any would have prevented the resurgence of HRV due to its neurotoxic effects. In fact, the outliers were the two youngest patients, who were operated when they were 22 and 28 years old, respectively, 3·5 and 2·7 years before our study. As nerve plasticity is more efficient in younger than in later life (Burke & Barnes, 2006; Kumar *et al.*, 2012; Kempsell & Fieber, 2015), we speculate that three years may have been enough, in these two patients, to recover afferent neural fibres between lungs and cardiorespiratory centres. It would be interesting to repeat the experiments on these patients in 2-4 years: if the hypothesis of a gradual reinnervation of transplanted lungs holds, we would expect a progressive increase in the power of the HF peak.

The HF peak of HRV was centred around a 0·30-Hz frequency in BLTR, while it was located around 0·20 Hz in controls, coherently with the finding that spontaneous BF was higher in BLTR than in controls. During cadenced breathing, the HF peak of HRV in controls was higher than during free breathing. These observations confirm that the HF peak is modulated by the breathing cycle mechanism, as pointed out by several authors (Malliani *et al.*, 1991; Taha *et al.*, 1995; Perini & Veicsteinas, 2003). By contrast, in BLTR, the power of the HF peak was extremely low, whether during free

Table 2 Mean and standard deviations of all parameters calculated by mean of heart rate variability, in supine position in the three investigated conditions.

HRV (*n* = 11)		**FB**	**CB**	**HG**
P_{tot} [ms2 Hz-1]	P	128·3 ± 144·3[a]	126·6 ± 118·3[a]	243·6 ± 229·8[a]
	C	1799·1 ± 1303·3	927·5 ± 448·5	1735·9 ± 947
LF [ms2 Hz-1]	P	86·1 ± 163·8[a]	37·3 ± 48·14[a]	133·2 ± 205[a]
	C	480 ± 377·6	221·5 ± 197·1	381 ± 237·8
HF [ms2 Hz-1]	P	30·5 ± 53·6[a]	11 ± 11·21[a]	57·8 ± 81·1[a]
	C	346 ± 230	663·9 ± 646·7	522·5 ± 508·2
LF/HF	P	3·2 ± 2·1[a]	2·5 ± 2·1[a]	3·6 ± 3·5[a]
	C	1·5 ± 1·3	0·9 ± 0·8	1 ± 0·9
LFnu [%]	P	69·8 ± 9·4[a]	61·3 ± 16·5[a]	62·1 ± 21·7[a]
	C	48·4 ± 20·1	38·9 ± 19·6	40 ± 19·8
HFnu [%]	P	29 ± 16·2[a]	35·5 ± 19·45[a]	26·07 ± 10·8[a]
	C	45 ± 16·4	55·5 ± 16·4	51·9 ± 19·3
HRV (*n* = 9)		**FB**	**CB**	**HG**
P_{tot} [ms2 Hz-1]	P	66·4 ± 52·2[a]	68·6 ± 61·6[a]	168·5 ± 147·8[a]
	C	1804·6 ± 932·8	932·8 ± 500·1	1597·9 ± 819·1
LF [ms2 Hz-1]	P	15·6 ± 13·9[a]	18·8 ± 21·63[a]	187·2 ± 312·3
	C	375·8 ± 295·7	224·4 ± 212·6	356 ± 211·8
HF [ms2 Hz-1]	P	7·4 ± 4·9[a]	5·3 ± 3·16[a]	11·2 ± 7·8[a]
	C	334·7 ± 236	741·6 ± 694·2	457·6 ± 499
LF/HF	P	3·3 ± 2·3[a]	2·7 ± 2·3[a]	3·1 ± 2·4[a]
	C	1·1 ± 0·7	1 ± 0·1	1·1 ± 0·9
LFnu [%]	P	63·5 ± 20·7[a]	58·2 ± 23·3[a]	65·1 ± 23·1[a]
	C	46·7 ± 20	39·2 ± 21·8	41·6 ± 21·8
HFnu [%]	P	29·4 ± 18[a]	33·2 ± 19·4[a]	24·4 ± 11·3[a]
	C	45·5 ± 14·8	54·1 ± 17·9	50·8 ± 21·3

Top panel, eleven patients and controls; Bottom panel, nine patients and controls; HRV, heart rate variability; P_{tot}, total power; LF, low-frequency power; HF, high-frequency power; LF/HF, low-frequency to high-frequency ratio; LFnu, normalized LF power; HFnu, normalized HF power; P, patients; C, controls; FB, free breathing; CB, cadenced breathing; HG, handgrip.
[a]significantly different from controls.

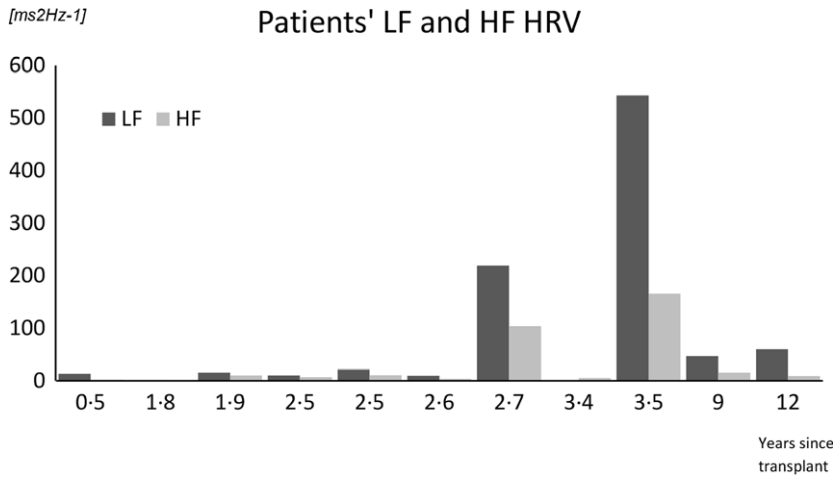

Figure 3 LF and HF power values for HRV during free breathing for every patient, from the most recent to the oldest surgery (6 months to 12 years). X axis is shortened for a better reading.

Table 3 Mean and standard deviations of all parameters calculated by mean of systolic arterial pressure, diastolic arterial pressure, mean arterial pressure at steady state, in supine position in the three investigated conditions.

		Free breathing	Cadenced breathing	Handgrip
SAP				
P_{tot} [ms2 Hz-1]	P	14·5 ± 11·4	7·8 ± 4·4[a]	18·1 ± 11·4
	C	24·7 ± 16·6	15·6 ± 8·9	18·7 ± 15·3
LF [ms2 Hz-1]	P	3·5 ± 3·2[a]	1·6 ± 1·1[a]	3·7 ± 2·9
	C	6·4 ± 3·8	4·4 ± 2·7	5·2 ± 3·1
HF [ms2 Hz-1]	P	2·0 ± 1·3	2·2 ± 1·9	3·6 ± 4
	C	1·2 ± 1·0	1·2 ± 1·1	2·0 ± 1·7
LF/HF	P	2·0 ± 1·8[a]	1·2 ± 1[a]	2·7 ± 2
	C	5·3 ± 3·5	3·8 ± 4·3	3·4 ± 2·9
DAP				
P_{tot} [ms2 Hz-1]	P	4·5 ± 3·7	2·6 ± 1·5[a]	7·8 ± 5·6
	C	6·9 ± 4	6·2 ± 4·6	9 ± 7·6
LF [ms2 Hz-1]	P	1·5 ± 1·5[a]	0·6 ± 0·7[a]	2·1 ± 1·9
	C	2·7 ± 1·6	1·9 ± 1·4	2·3 ± 1·2
HF [ms2 Hz-1]	P	1·1 ± 1·4	0·7 ± 0·5	2·1 ± 2·7
	C	1·1 ± 1	1·3 ± 1·3	1·6 ± 2·3
LF/HF	P	1·8 ± 1·4[a]	1·4 ± 1·4[a]	2·4 ± 3·5
	C	4·1 ± 3·2	3 ± 2·8	2·6 ± 1·5
MAP				
P_{tot} [ms2 Hz-1]	P	5·5 ± 3·5	3·5 ± 1·8[a]	8·6 ± 5·1
	C	7·5 ± 4·2	5·4 ± 2·2	8·3 ± 5
LF [ms2 Hz-1]	P	1·5 ± 1·2[a]	0·8 ± 0·7[a]	2·8 ± 3·5
	C	2·8 ± 1·5	1·9 ± 1·1	2·8 ± 1·7
HF [ms2 Hz-1]	P	0·8 ± 0·6	0·9 ± 0·7	1·9 ± 2·1
	C	0·5 ± 0·4	0·6 ± 0·7	1 ± 1·3
LF/HF	P	2·2 ± 1·8[a]	1·4 ± 1·3[a]	2·7 ± 2·7[a]
	C	9·3 ± 9	6 ± 6·7	5·4 ± 4·1

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; P_{tot}, total power; LF, low-frequency power; HF, high-frequency power; LF/HF, low-frequency to high-frequency ratio; LFnu, normalized LF power; HFnum, normalized HF power; P, patients; C, controls.
[a]significantly different from controls.

breathing or during cadenced breathing, more than 100 times lower than in controls. This is compatible with the notion that lung denervation suppressed most of the HF peak.

Table 4 Parameters calculated by means of heart rate variability, in supine position in the three investigated conditions, for the two outliers and respective control counterparts.

HRV		Patient 6	Control 6	Patient 10	Control 10
P_{tot} [ms2 Hz-1]	FB	1338·8	1942·4	503·9	1606·7
	CB	1049·6	866·1	272·2	941·8
	HG	774·1	3509·9	389·4	1204·1
LF [ms2 Hz-1]	FB	542·9	725·2	219·2	322·7
	CB	155·7	93·4	85·6	323·2
	HG	302·6	789·9	121·8	197·3
HF [ms2 Hz-1]	FB	165·8	200·3	103·9	592·8
	CB	260·4	193·2	37·9	435·5
	HG	236·9	1251·5	73·5	377·4
LF/HF	FB	3·3	3·6	2·1	0·5
	CB	0·6	0·5	2·3	0·7
	HG	1·3	0·6	1·7	0·5
LFnu [%]	FB	76·1	76·8	67·1	35·1
	CB	36·8	31·9	66·3	42·5
	HG	44·9	32·8	52·5	32·3
HFnu [%]	FB	23·3	21·2	31·8	64·4
	CB	61·6	66·0	29·4	57·3
	HG	35·1	52·0	31·7	61·8

HRV, heart rate variability; P_{tot}, total power; LF, low-frequency power; HF, high-frequency power; LF/HF, low-frequency to high-frequency ratio; LFnu, normalized LF power; HFnu, normalized HF power; FB, free breathing; CB, cadenced breathing; HG, handgrip.

Also, the LF power was smaller in BLTR than in controls, but not as much as the HF power. During handgrip, no difference between BLTR and controls was found, whereas in free and cadenced breathing, the LF power was some 10 times smaller in BLTR than in controls. This suggests that the LF peak was less affected than the HF peak by the breathing patterns.

Although the hypothesis that a neural modulation from lung afferents determines most of the HF component of HRV is essentially supported by the present results, there remains a component of spontaneous HRV that must be related to different mechanisms. We speculate that oscillations in heart filling,

and thus in central venous pressure, following changes in lung volumes may stimulate atrial low-pressure cardiac baroreceptor. This might correct the oscillations in central venous pressure synchronous with changes in lung volume by means of compensatory heart rate oscillations, whence a surviving LF and HF component in BLTR.

Concerning spontaneous variability of SAP and DAP, differences between BLTR and controls were smaller than for HRV. The HF power did not differ between BLTR and controls in any condition. No differences between patients and controls were observed during handgrip for any of the investigated parameters. This means that blood pressure variability was poorly affected, much less than HRV, by lung denervation. The LF power of SAP is linked to peripheral sympathetic modulation (Pagani *et al.*, 1997). Notwithstanding, the LF/HF ratio was higher, and consistently, LFnu was higher and HFnu lower in BLTR than in controls, suggesting predominant sympathetic modulation of HR in patients. Thus, the lower LF power of SAP in BLTR would confirm the dissociation between modulation mechanisms involved in arterial pressure variability, representing the peripheral oscillatory component, and HRV, representing the cardiac oscillatory component. We speculate that the lung afferents to the cardiovascular centres affect specifically the central (heart) component of cardiovascular modulation by the ANS. Yet we acknowledge that meaning and links between LF and fluctuation in the modulation of the ANS are highly disputed in literature (Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Eckberg, 1997; Malliani *et al.*, 1998; Notarius *et al.*, 2001; Billman, 2011, 2013), so that some authors (Eckberg, 1997; Goldstein *et al.*, 2011; Billman, 2013; Heathers, 2014) proposed that the LF power is a poor indicator of sympathetic heart modulation.

The analysis of HRV is a potentially useful tool in different clinical settings (La Rovere *et al.*, 2003). Cardiac autonomic dysfunction changes HRV and is a risk factor of cardiovascular diseases (Pipilis *et al.*, 1991; Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). An evaluation of cardiac autonomic dysfunction by HRV helps predicting cardiac issues. Low HRV is, for example, associated with higher risk of heart failure (Tuininga *et al.*, 1994; Sandercock & Brodie, 2006), sudden cardiac death (Kudaiberdieva *et al.*, 2007) and mortality after myocardial infarction (Wolf *et al.*, 1978; Kleiger *et al.*, 1987; Dougherty & Burr, 1992). In the case of BLTR, atherosclerotic diseases are potentially accelerated by immunosuppressive therapy (Kasiske, 1988; Hruban *et al.*, 1990). Then, coronary artery diseases are not uncommon in BLTR (Reed *et al.*, 2012; Castleberry *et al.*, 2013).

The remarkable differences between BLTR and controls imply that HRV analysis may represent a sensitive tool for assessing autonomic improvements in BLTR follow-up. For example, exercise training, improving exercise capacity also in BLTR (Wickerson *et al.*, 2010), carries along an increased vagal modulation of the heart (Levy *et al.*, 1998), which may be reflected by a decrease in the LF/HF ratio. Moreover, progressive heart reinnervation after surgery may translate into an increased P_{tot}, LF and HF of HRV, making the BLTR spectra closer to those of healthy subjects. Under this respect, a stimulating perspective is opened by the two young patients who showed P_{tot} values more than two standard deviations higher than the group mean. These patients were the two youngest investigated BLTR and also the youngest at the time of surgery. A follow-up study on young BLTR from operation would inform on whether indexes of HRV progress towards normalization as time after operation goes on.

Concerning BRS, we observed a drastic reduction in BLTR with respect to controls in every condition. Autonomic alterations with reduced vagal or increased sympathetic nerve activity are always accompanied by decreased BRS (Billman *et al.*, 1982; Schwartz *et al.*, 1988; La Rovere *et al.*, 2001). This is especially true during exercise (Perini & Veicsteinas, 2003). The reduced BRS is therefore consistent with the observed increase in LFnu in BLTR. As physical training increases BRS (La Rovere *et al.*, 2002), it would be relevant for BLTR to systematically enter a training programme and useful for clinical teams to exactly know how they respond to it.

A limitation of this study concerns the fact that this is a transversal study, wherein we compared BLTR to healthy subjects, instead of a longitudinal study, with the same patients being studied before and after BLT. Thus, we acknowledge that the attribution to lung denervation of the observed effects of HRV and BRS was indirect, not direct: performance of a longitudinal study would be needed to further reinforce the present interpretation. Another point is the transplant medication that can cause side effects on both the central and peripheral nervous system. For example, tacrolimus, used on nine of our eleven patients, is well known to have neuropathies associated with it. Thus, we cannot totally exclude that the lack of HRV may be due to a neurotoxic effect of tacrolimus. Nevertheless, the fact that the two outliers were also under tacrolimus speaks against this second hypothesis.

Conclusion

From a physiological viewpoint, lung denervation carried along an important reduction in HRV, indicating that neural modulation from lung afferents contributes largely to HRV. The higher LF/HF ratio implies that the P_{tot} drop was mostly due to the HF component. In contrast, the effects of lung denervation on blood pressure variability were much smaller than on HRV. This suggests that the effects of lung denervation were specific to HRV modulation and confirms that blood pressure variability and HRV are under different control mechanisms.

Clinically speaking, it is very important for physicians to be aware that BLTR have a decreased cardiac autonomic regulation with a global and remarkable reduction in all parameters related to HRV spectral analysis. Those patients need an appropriate training programme to be strengthened, and it would be very

useful to quantify exactly how they respond to it. Further research could evaluate HRV indices as sensitive tool for assessing autonomic improvements in BLTR follow-up.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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