ORIGINAL ARTICLE

Body composition and maximal exercise capacity after heart transplantation

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Abstract

Aims Maximal exercise capacity as measured by peak oxygen consumption (pVO_2) in cardiopulmonary exercise testing (CPET) of heart transplant recipients (HTR) is limited to a 50–70% level of healthy age-matched controls. This study investigated the relationship between body composition and pVO_2 during the first decade post-transplant.
Methods and results Body composition was determined by dual-energy X-ray absorptiometry (DXA) and pVO_2 by CPET in 48 HTR (n = 38 males; mean age 51 ± 12 years). A total of 95 assessments were acquired 1–9 years post-transplant, and the results of four consecutive periods were compared [Period 1: 1–2 years (n = 25); 2: 3–4 years (n = 23); 3: 5–6 years (n = 23); 4: 7–9 years (n = 24)]. Linear regression analysis analysed the correlation between pVO_2 and pairs of appendicular lean mass (ALM) and fat mass (FM). The relation between ALM and daily dose of calcineurin inhibitor (CNI) was explored using partial correlation controlling for age, gender, and height. pVO_2 increased from 0.98 (0.34) to 1.35 (0.35) L/min ($P < 0.01$) between Periods 1 and 4 corresponding to 54.5–63.3% of predicted value. Peak heart rate (HR) raised from 115 ± 19 to 131 ± 23 b.p.m. ($P = 0.05$), and anaerobic threshold (AT = VO_2 achieved at AT) increased from 0.57 (0.18) to 0.83 (0.35) L/min ($P < 0.01$) between Periods 1 and 3. Median FM normalized to height^2 (FMI) always remained elevated (>8.8 kg/m^2). ALM normalized to body mass index increased from 0.690 (0.188) to 0.848 (0.204) m^2 ($P = 0.02$) between Periods 1 and 4, explaining 45% of the variance of pVO_2 ($R^2 = 0.455$; $P < 0.001$). Eighty-one per cent of the variance of pVO_2 ($R^2 = 0.817$; $P < 0.001$) in multiple regression was explained by AT ($\beta = 0.488$), ALM ($\beta = 0.396$), peak HR ($\beta = 0.366$), and FMI ($\beta = -0.181$). ALM was negatively correlated with daily CNI dose (partial $R = -0.258$; $P = 0.01$).
Conclusions After heart transplantation, the beneficial effect of peripheral skeletal muscle gain on pVO_2 is opposed by increased FM. Our findings support lifestyle efforts to fight adiposity and CNI dose reduction in the chronic stable phase to favour positive adaptation of peripheral muscle mass.

Introduction

Exercise capacity increases largely within the first 6 post-operative months after heart transplantation (HTx), and further improvement up to 2 years post-transplant has been reported.[1] However, most exercise studies indicate a persistent limitation of peak oxygen consumption (pVO_2) to 50–70% of the predicted values for age-matched healthy controls in the late phase after HTx.[2–5] Reported predictors of pVO_2 as assessed by cardiopulmonary exercise testing (CPET) in HTx recipients with normal systolic left ventricular function are age, peak heart rate, β_1-adrenergic receptor polymorphism, diastolic function, body mass index (BMI), and body fat percentage.[5–8]

Intuitively, exercise capacity after HTx should improve with increase of skeletal muscle mass. But respective studies were not conclusive because of small cohorts, short follow-up, and non-quantitative assessment of peripheral skeletal muscle mass.[9–11] Today, magnetic resonance imaging (MRI) and computed tomography (CT) are considered gold standards to quantify muscle mass because they almost perfectly correlate with cadaveric values.[12] However, the time required for MRI and its contraindication by metal implants such as abandoned leads from pre-transplant ICD implantation, the radiation exposure associated with CT, as well as the cost and the need for an expert team to analyse data have limited their use in clinical research. In contrast, dual-energy X-ray absorptiometry (DXA) offers the advantages of very low radiation exposure (~0.01 mSv) and easy use and accessibility for a reduced cost. Moreover, this two-dimensional imaging technique is able to measure lean mass and fat mass (FM) with high precision and strong correlation with MRI (correlation coefficient $r = 0.88$–0.97).[13–15]

This study therefore applied DXA for quantification of peripheral skeletal muscle mass and body FM in order to investigate their respective impact on pVO_2 after HTx. The study design was monocentric and observational and based on a follow-up protocol that was similar for all study participants.

Methods

Study population

Forty-eight HTx recipients with transplant operation between 2008 and 2018 presenting for their annual post-transplant follow-up visit at the Lausanne University Hospital (CHUV) were included after giving written informed consent to participate. All participants underwent a 3 week exercise-based cardiac rehabilitation programme in a specialized clinic at discharge from the hospital following their HTx and were stimulated to perform regular exercise thereafter. The study was approved by the local ethic committee (CER-VD No. 2018-01719) and complied with the Declaration of Helsinki. Study flow chart and baseline characteristics of the cohort are summarized in Supporting Information, *Figure S1* and *Table S1*. Out of 111 body composition (BC) collected between August 2016 and January 2020, 95 were included in the analysis (see Supporting Information, *Figure S1* for details).

Body composition and cardiopulmonary exercise testing

The time interval between BC and CPET had to be <6 months and was <3 months for 92/95 BC–CPET couples [median time: 0 days, interquartile range (IQR) = 3].

Dual-energy X-ray absorptiometry scans (Lunar iDXA, GE Healthcare, Madison, WI, USA) were performed to assess appendicular lean mass (ALM), FM, and visceral adipose tissue (VAT). Body weight and height were measured just before the acquisition. ALMI (appendicular lean mass index) was calculated as $ALM/height^2$ and ALM% as $(ALM/weight)*100$. FMI (fat mass index) was calculated as $FM/height^2$, and FM% as $(FM/weight)*100$.

Measures of ALM standing in the sarcopenic range were identified by applying the gender-specific cut-off values from two different consensus definitions of sarcopenia based respectively on ALMI (IWGS 2011[16]) and ALM/BMI (FNIH 2014[17]). Considering reported discrepancies in measurements of ALM between manufacturers, ALMI measures were also classified as low if standing below the 20th percentile of the gender-specific and age-specific reference value obtained in a healthy Caucasian population with DXA scans of the same manufacturer as in the present study.[18] Sarcopenic obesity was identified by the presence of an ALMI value in the sarcopenic range in addition to a BMI > 30 kg/m^2, or a value of FM% above a gender-specific cut-off (male >27%, female >38%) as previously applied in former studies.

Maximal exercise parameters were obtained by symptom-limited incremental exercise testing applying an individualized ramp protocol performed on an electrically braked cycloergometer (Ergoline 900/911 digital, Ergoline GmbH, Germany).[8] A respiratory exchange ratio (RER) ≥ 1.1 at peak exercise was considered as a maximal test. Respiratory gas exchange was measured breath by breath.

Peak oxygen consumption was defined as the highest VO_2 achieved over the last 30 s of each test and reported in absolute value (pVO_2_abs), normalized to body weight (pVO_2_kg), and in per cent of predicted VO_{2max} ($pVO_2\_\%$). Prediction of VO_{2max} was based on the Wassermann and Hansen equations accounting for gender, age, height, weight, and predicted weight.[19,20]

The ventilatory anaerobic threshold (AT) was determined by the V-slope method and reported in per cent of predicted VO_{2max} [= AT (%)] and in VO_2_abs or VO_2_kg achieved at AT (= AT_abs or AT_kg).

Statistical analysis

The 'ALL-dataset' included all 95 BC–CPET pairs. Clinical characteristics, immunosuppressive regimen (CS = corticosteroids, CNI = calcineurin inhibitor), laboratory values [haemoglobin (Hb), ferritin, creatinine, estimated glomerular filtration rate (eGFR), N-terminal pro-brain natriuretic peptide (NT-proBNP)], echocardiographic parameters, invasive haemodynamic parameters, and vasculopathy grading on the basis of routine coronary angiography were collected from the patient's electronic chart at the Lausanne University Hospital. The time from HTx classified each BC/CPET pair into the

following periods: (1) 1–2 years, (2) 3–4 years, (3) 5–6 years, and (4) 7–9 years post-transplant, with similar sample size in each period (*Table 1*).

Because the data collection period extended over a period of 43 months, several participants contributed one to four times to the ALL-dataset (Supporting Information, *Figure S1*). Each measure was considered as a single event even when being consecutive. To control for disproportionated contribution of individual patients with >1 BC and CPET assessment, the 'COUPLE-dataset' was set up containing 48 individual BC–CPET couples chosen to obtain similar sample size in each period (Supporting Information, *Table S7*). Finally, a paired analysis ('PAIRED-analysis') of BC findings was performed in seven participants (*n* = 5 male) who underwent three consecutive BC at Years 1, 2, and 3 after HTx.

Continuous variables were presented as mean ± standard deviation or median (IQR) according to their distribution assessed by the Shapiro–Wilk normality test. Categorical variables were presented as absolute count and percentage. One-way ANOVA or the Kruskal–Wallis test compared means or medians between periods. By significant result, differences were further explored with a post hoc test (Tukey's honestly significant difference, Games–Howell, or pairwise post hoc test), and proportions were compared with a χ^2 test or

Fisher's exact test, as appropriate. Comparisons of means between Years 1, 2, and 3 in the PAIRED-analysis were assessed with a one-way ANOVA repeated measurement.

The relation between CNI daily dose and ALM was explored using partial correlation controlling for age, gender, height, and FM.

The relations between pVO_2 and other variables were explored with bivariate correlations (Pearson's correlation coefficient). Analyses were run for pVO_2_kg and for pVO_2_abs to suppress the influence of the relation between the normalizing factor 'weight' in pVO_2_kg and the variable tested. Variables showing at least moderate correlation with pVO_2_abs ($R \geq 0.3$; $P < 0.05$) were further tested individually by simple linear regression, and together in a multiple regression analysis applying a stepwise approach (inclusion/exclusion criteria: probability of F to enter ≤0.05, probability of F to remove >1). Strong correlations ($R \geq 0.7$) between predictors in the final model were excluded to prevent multicollinearity. Both the ALL-dataset and the COUPLE-dataset were compared to check for concordant trends of results. A P-value < 0.05 was considered statistically significant for all analyses. All statistical analyses were performed using SPSS Statistics (Version 26; IBM; USA). Figures were generated by GraphPad Prism (Version 9).

Table 1 Baseline characteristics by period after heart transplantation

	Period after HTx								
	Years 1–2		Years 3–4		Years 5–6		Years 7–9		
	Median, mean, N	(IQR), ±SD, (%)	Median, mean, N	(IQR), ±SD, (%)	Median, mean, N	(IQR), ±SD, (%)	Median, mean, N	(IQR), ±SD, (%)	P^a
Number of BCs	25	(100%)	23	(100%)	23	(100%)	24	(100%)	—
Male gender	14	(56%)	18	(78.3%)	18	(78.3%)	21	(87.5%)	0.07
Age at exam (years)	54	(18)	59	(11)	57	(20)	58	(31)	0.2
BMI[b] (kg/m²)	23.9	(7.1)	26.2	(6.9)	26.0	(6.1)	26.8	(7.4)	0.5
Donor age (years)	57a	(16)	43	(37)	43a	(25)	44	(22)	0.02
Male donor	11	(44%)	12	(52.2%)	14	(60.9%)	19	(79.2%)	0.07
Ischaemic time (min)	169	(35)	180	(75)	193	(85)	189	(64)	0.04
CNI (%)[c]	111.0a,b	(51.1)	73.5	(32.9)	58.8a	(45.6)	58.8b	(44.8)	<0.01
CyA (mg/kg/day)	3.4a,b	(1.2)	2.8a	(1.0)	2.8b	(1.5)	2.3	(1.0)	<0.01
FK (mg/kg/day)	0.09	(0.08)	0.05	(0.04)	0.03	(0.04)	0.04	(0.06)	0.07
Prednisone	14	(56%)	2	(8.7%)	1	(4.3%)	2	(8.3%)	<0.01
Daily dose (mg/kg)	0.06	±0.04	0.07	±0.02	0.07	—	0.03	—	—
Vit. D3 800 IU/day	24	(96%)	20	(87%)	18	(78.3%)	21	(87.5%)	0.3
Beta-blocker	4	(16%)	7	(30.4%)	7	(30.4%)	9	(37.5%)	0.3
% of target dose	12.5	(9.3)	25	(25)	25	(93)	25	(25)	0.6
NDHP-CCB	3	(12%)	1	(4.3%)	8	(34.8%)	3	(12.5%)	0.05
% of max. dose	50	(0)	33.3	—	66.7	(12.5)	50		0.06
Statin	20	(80%)	16	(69.6%)	18	(78.3%)	21	(87.5%)	0.5
% of max. dose[d]	12.5	(18.7)	12.5	(9.3)	12.5	(12.5)	12.5	(12.5)	0.2

BC, body composition; BMI, body mass index; CNI, calcineurin inhibitor; CyA, cyclosporine A; FK, tacrolimus; IQR, interquartile range; NDHP-CCB, non-dihydropyridine calcium channel blocker; SD, standard deviation.
[a]Comparisons of proportions, medians, or means between periods. Values in the same row sharing the same subscript (a) were significantly different (*P* < 0.05 post hoc).
[b]Median weight and height were not significantly different between periods.
[c]Per cent of maximal theoretic daily dose (CyA = 4 mg/kg/day, FK = 0.075 mg/kg/day).
[d]Equivalent of 80 mg atorvastatin.

Results

Baseline characteristics by period after heart transplantation

The number of BC investigations was equally distributed between periods. The proportion of men was higher in the total cohort (74.5%) (Supporting Information, *Table S1*) and in each period (*Table 1*). Ischaemic time was shorter in study participants in Periods 1 and 2. Donor age was higher in Period 1. BMI was not significantly different between periods (range: 23.9–26.8 kg/m², $P = 0.5$). Most patients were on oral supplementation of Vitamin D3 (800 IU/day) for the prevention of osteoporosis, and statin therapy for the prevention of cardiac allograft vasculopathy, without significant differences between periods ($P = 0.3$, range: 87–93% and $P = 0.5$, range: 69–87%, respectively). The proportion of assessments performed on non-dihydropyridine calcium channel blockers was higher by trend at Period 3, with a trend towards higher dose (*Table 1*).

Body composition

Skeletal muscle mass

There was a progressive increase in ALM normalized to height² (ALMI) and BMI (ALM/BMI) from Periods 1 to 4 [6.03 (2.08) to 7.41 (1.39) kg/m², $P = 0.03$ and 0.690 (0.188) to 0.848 (0.204) m², $P = 0.02$] (*Figure 1*, Supporting Information, *Table S2*).

More than 20% of ALM measures in each period were in the sarcopenic range (*Table 2*). This proportion did not decrease significantly from Period 1 to later periods (range: 29–44%, $P = 0.6$ and 21–28%, $P = 0.9$ based respectively on

Figure 1 Body composition by period after HTx. ALM, appendicular lean mass; ALMI, appendicular lean mass index; BMI, body mass index; FM, fat mass; FMI, fat mass index; HTx, heart transplantation.
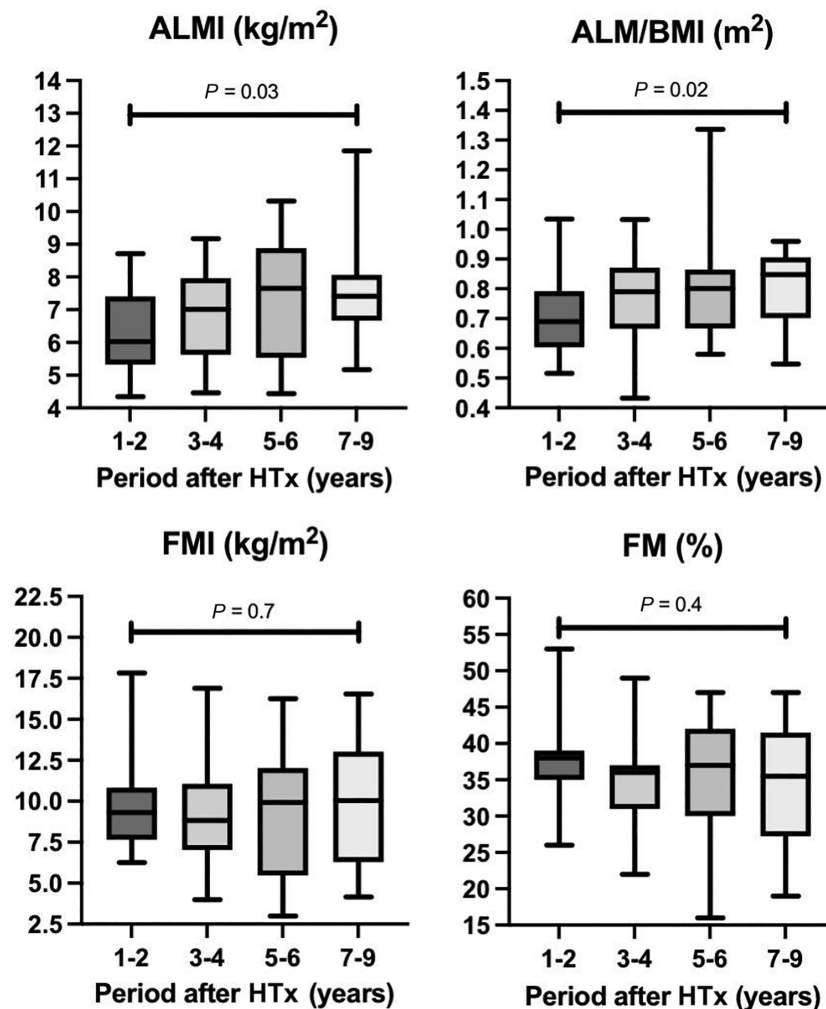
Table 2 Proportions of sarcopenia or appendicular lean mass index measures in the lower range

	Period after HTx								
	Years 1–2		Years 3–4		Years 5–6		Years 7–9		
	N	(%)	N	%	N	%	N	%	P^a
Sarcopenia[b]									
IWGS 2011[16] (ALMI[c])	11	(44%)	11	(47.8%)	9	(39.1%)	7	(29.2%)	0.6
FNIH 2014[17] (ALM/BMI[d])	7	(28%)	6	(26.1%)	5	(21.7%)	6	(25%)	0.9
ALMI in the lower range									
ALMI <20th percentile[e]	20	(80%)	15	(65.2%)	13	(56.5%)	13	(54.2%)	0.2
Sarcopenic[b] obesity									
Low ALMI[c] + BMI > 30 kg/m²	0	(0%)	0	(0.0%)	0	(0%)	0	(0%)	—
Low ALMI[c] + high FM%[f]	6	(24.0%)	7	(30.4%)	6	(26.1%)	1	(21.1%)	0.08

ALM, appendicular lean mass; ALMI, appendicular lean mass index; BMI, body mass index; FM, fat mass; HTx, heart transplantation.
[a]Comparisons between periods.
[b]Based exclusively on the measure of ALM: muscle strength and gait speed were not assessed.
[c]Male <7.23 kg/m², female <5.67 kg/m².
[d]Male <0.789 m², female <0.512 m².
[e]Of the sex-specific and age-specific reference value measured in a healthy Caucasian population.[18]
[f]Male >27%, female >38%.

ALMI and ALM/BMI). However, 80% of ALMI measures in Period 1 compared with 54% in Period 4 (P = 0.2) stand below the 20th percentile of the reference value obtained in a reference Caucasian population.[18] The proportion of BC assessments at each period compatible with sarcopenic obesity largely depended on which definition of obesity was applied, ranging from 0% at each period based on BMI to 21–30% based on FM%, without significant difference between periods (P = 0.08) (*Table 2*).

Appendicular lean mass increased with progressive distance to transplant operation, and this increase was negatively correlated with daily CNI dose when adjusted for gender, age, and height (partial R = −0.258; P = 0.01). The decrease of daily CNI dose as a function of Periods 1 to 4 is illustrated in *Table 1*.

No correlation was found between ALM and prednisone dose, but only 20% of all BC investigations were performed on CS therapy, the majority of which during Period 1 (*Table 1*). By trend, the ALM/FM ratio was lower in study participants on CS treatment compared with those who were weaned [0.68 (0.12) vs. 0.75 (0.27); P = 0.06], which was not explained by a difference in gender distribution (male: 73.7% vs. 75.0%, P = 1.0).

Fat mass

Median FMI always remained elevated >8.8 kg/m² at each period (*Figure 1*, Supporting Information, *Table S2*). Eighty-four per cent of BC investigations acquired in males and 54% of those in females classified in the range of excess fat when considering the cut-off value of ≤6 kg/m² for males and ≤9 kg/m² for females.[21] Higher FMI was found in women treated with CS compared with those who were weaned [10.3 (6.0) vs. 8.7 (2.4) kg/m²; P = 0.03]. In the PAIRED-analysis only, FMI significantly decreased between 1 and 3 years post-transplant (10.0 to 8.2 kg/m²; P < 0.001) (Supporting Information, *Figure S2*).

Biological characteristics and cardiac allograft function

Mean Hb was higher in Periods 3 and 4 (134 ± 15 g/L; 138 ± 18 g/L) compared with Period 1 (121 ± 14 g/L) (P < 0.01) (*Table 3*).

Median left ventricular ejection fraction (LVEF) was always within the normal range; right ventricular (RV) function was either preserved or mildly impaired (Supporting Information, *Table S4*). Mean cardiac index (CI) and median pulmonary capillary wedge pressure (PCWP) were in the normal range (Supporting Information, *Table S5*). By trend, more study participants had cardiac allograft vasculopathy (CAV) ISHLT grade[22] > 1 in Period 4 compared with Period 1 (17.4% vs. 0%; P = 0.2) (Supporting Information, *Table S6*).

Cardiopulmonary exercise testing

A total of 80/95 were maximal based on a RER at exercise peak ≥1.1; RER of one test was <1.0. The overall pVO$_2$_% was 60.5 ± 14.5%.

Peak HR (%) significantly increased from Periods 1 to 2 (69.5 ± 11.7% to 79.9 ± 14.6%; P < 0.01) but not thereafter. Mean pVO$_2$_% increased by trend from Periods 1 to 3 [54.5 ± 14.6% to 64.2 ± 13.5%; mean difference 9.7%; 95% confidence interval (CI) −1.0 to 20.4; P = 0.09] (*Figure 2*, Supporting Information, *Table S3*). Median O$_2$ pulse was below 90% of the predicted value at each period without significant differences. The VE/VCO$_2$ slope improved after HTx (P = 0.03) with decrease from 35.0 ± 5.0% to 30.0 ± 6.0% between Periods 1 and 3 (P = 0.01). AT (%) improved through years after HTx (P = 0.02) to reach its best values in Period 3. Despite of all, median AT (%) remained low (<40%) even years after HTx suggesting a low level of training.

Table 3 Laboratory values by period after heart transplantation

	Period after HTx							
	Years 1–2		Years 3–4		Years 5–6		Years 7–9	
	Median, mean	(IQR), SD	Median, mean	(IQR), SD	Median, mean	(IQR), SD	Median, mean	(IQR), SD	P^a
Haemoglobin (g/L)	121[a,b]	±14	126	±15	134[a]	±15	138[b]	±18	<0.01
Ferritin (μg/L)	137	(169)	295	(317)	157	(137)	114	(54)	0.09
Creatinine (μM)	111	(61)	132	(38)	133	(62)	134	(37)	0.4
eGFR (mL/min)	52	(19)	47	(10)	44	(24)	44	(12)	0.2
NT-proBNP (ng/L)	789	(608)	453	(798)	198	(396)	632	(907)	0.09

eGFR, estimated glomerular filtration rate; HTx, heart transplantation; NT-proBNP, N-terminal pro-brain natriuretic peptide; SD, standard deviation.
[a]Comparisons between periods. Values in the same row sharing the same subscript ([a,b]) are significantly different at $P < 0.05$ (post hoc).

Figure 2 Cardiopulmonary exercise testing findings by period after HTx. HR, heart rate; HTx, heart transplantation; pVO_2, peak oxygen consumption.
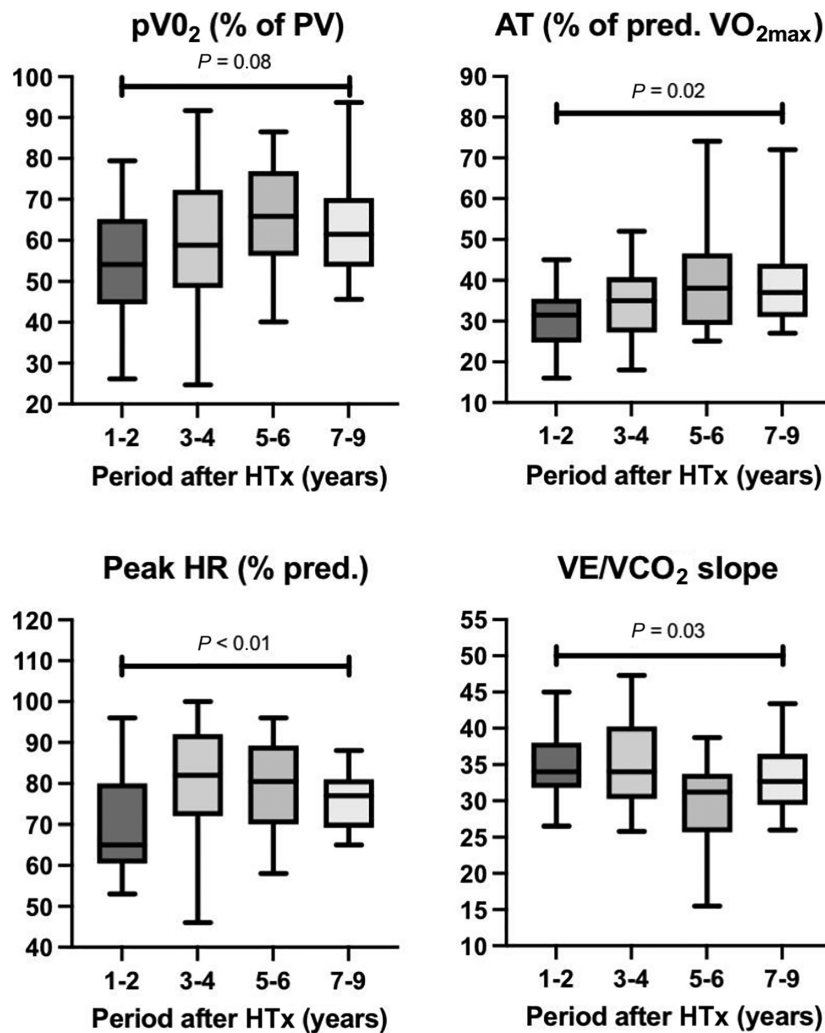
Impact of body composition and other variables on peak oxygen consumption

In univariate analysis, pVO$_2$_abs was positively related with male gender, years after HTx, ALM/BMI, height, weight, peak HR, AT_abs, Hb, and eGFR. There was a negative correlation of pVO$_2$_abs with age, donor age, FM%, VE/VCO$_2$, NT-proBNP, and ferritin (*Table 4a*). ALM/BMI shared the strongest positive correlation with pVO$_2$_abs (*R* = 0.675; *P* < 0.001) among all anthropometric variables tested,

Table 4a Univariate predictors of pVO$_2$_abs

Variable	N	R	R^2	Constant	B	95% CI	P
Age at BC (years)	95	−0.349	0.122	1.825	−0.110	−0.017 to −0.005	0.001
Donor age (years)	95	−0.354	0.125	1.608	−0.008	−0.013 to −0.004	<0.001
Years after HTx	95	0.372	0.139	0.955	0.060	0.029 to 0.090	<0.001
Male gender	95	0.424	0.180	0.941	0.383	0.215 to 0.552	<0.001
Weight (kg)	95	0.320	0.102	0.702	0.007	0.003 to 0.011	0.002
Height (cm)	95	0.545	0.296	−3.229	2.641	1.803 to 3.479	<0.001
ALM (kg)	95	0.567	0.321	0.417	0.04	0.028 to 0.052	<0.001
ALM%	95	0.633	0.401	−0.812	0.076	0.057 to 0.094	<0.001
ALMI (kg/m^2)	95	0.468	0.219	0.386	0.120	0.073 to 0.166	<0.001
ALM/BMI (m^2)	95	0.675	0.455	−0.110	1.722	1.334 to 2.110	<0.001
ALM/FMI (m^2)	95	0.548	0.301	0.747	0.202	0.138 to 0.265	<0.001
FM%	95	−0.346	0.120	1.924	−0.020	−0.031 to −0.009	0.001
Peak HR (b.p.m.)	95	0.489	0.239	0.108	0.009	0.006 to 0.012	<0.001
AT_abs (L/min)	83	0.810	0.657	0.213	1.391	1.168 to 1.613	<0.001
VE/VCO$_2$	89	−0.552	0.305	2.499	−0.038	−0.050 to −0.026	<0.001
Hb (g/L)	95	0.379	0.144	0.091	0.009	0.004 to 0.013	<0.001
Ferritin (μg/L)	49	−0.460	0.211	1.547	−0.001	−0.002 to −0.001	0.001
NT-proBNP (ng/L)	65	−0.430	0.185	1.424	−0.001	−0.002 to −0.008	<0.001
eGFR (mL/min)	95	0.313	0.098	0.748	0.010	0.003 to 0.016	0.003

ALM, appendicular lean mass; ALMI, appendicular lean mass index; BC, body composition; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; FM, fat mass; FMI, fat mass index; Hb, haemoglobin; HR, heart rate; NT-proBNP, N-terminal pro-brain natriuretic peptide.
Only variables sharing a significant correlation with pVO$_2$_abs were tested (Pearson's $R > 0.3$; $P < 0.05$).

explaining 45% of its variance in a simple linear regression ($R^2 = 0.455$; $P < 0.001$) (*Figure 3*). FM% had a significant negative correlation with pVO$_2$_abs ($R = −0.346$; $P = 0.001$). No haemodynamic or echocardiographic measurements at rest correlated with pVO$_2$_abs with an $R \geq 0.3$. Likewise, longer ischaemic time or CAV ISHLT grade > 1 were not associated with lower pVO$_2$_abs.

Among all significant predictors in the univariate analysis (*Table 4a*), only AT_abs, ALM/BMI, and peak HR (b.p.m.) remained independent predictors of pVO$_2$_abs in stepwise multiple regression ($R^2 = 0.803$; $P < 0.001$). Because BMI and FMI were strongly correlated ($R = 0.9$; $P < 0.001$), ALM and FMI (instead of ALM/BMI) were tested together with other predictors in multiple regression analysis. In this analysis, ALM and FMI remained significant independent predictors of pVO$_2$_abs with AT_abs and peak HR, accounting for more than 80% of its variance ($R^2 = 0.817$; $P < 0.001$) (*Table 4b*). The analysis of the COUPLE-dataset confirmed these findings (Supporting Information, *Tables S7–S10a*), with ALM being even the strongest independent predictor of pVO$_2$_abs before AT_abs, peak HR, and FMI, predicting 85% of its variance ($R^2 = 0.855$; $P < 0.001$) (Supporting Information, *Table S10b*).

Discussion

This observational study shows that ALM increased late after HTx. The increase of ALM correlated with the decrease of CNI daily dose. In contrast, FM remained for the most part unchanged even at large distance to HTx and despite of early withdrawal of CS in most study participants. ALM and FM were independent predictors of pVO$_2$ but with opposing effects explaining why pVO$_2$ continued to increase up to 6 years post-transplant in this study cohort.

In the study population, ALM increased despite of a rehabilitation limited to a 3 week programme early after HTx and an overall sedentary lifestyle of the study participants. The difference of ALM between Periods 1 and 4 was small, but ALM normalized to height^2 (ALMI) as well as BMI (ALM/BMI) showed a significant gain of ALM excluding that increase of ALM was related to increase in BMI as reported from a Caucasian reference population.[23] A similar increase in peripheral skeletal mass was also reported from HTx recipients remaining in a structured training programme for almost 2 years after transplant surgery.[9] The increase in the latter patients was ascribed to a training effect; however, our finding suggests that non-exercise-related factors should also play role.

Furthermore, CS were progressively withdrawn during the first 2 years after HTx in the majority of our study participants while few patients remained on low-dose prednisone thereafter. CS are known to promote both adipogenesis and muscle atrophy[24] suggesting that weaning may have favourable effects. Indeed, the ALM/FM ratio measured in the study participants weaned from CS tended to be higher, suggesting a clinical effect.

Calcineurin inhibition may be another exercise-independent factor explaining this increase in ALM because high dose of CNI was shown in animal models to prevent muscle regrowth or hypertrophy with a more prominent effect in slow-fibre muscle.[25,26] In the present study cohort, CNI dose was inversely correlated with ALM suggesting that

Figure 3 Simple regression analyses for the outcome pVO$_2$_abs or pVO$_2$_kg. ALM, appendicular lean mass; BMI, body mass index; FM, fat mass; pVO$_2$, peak oxygen consumption.
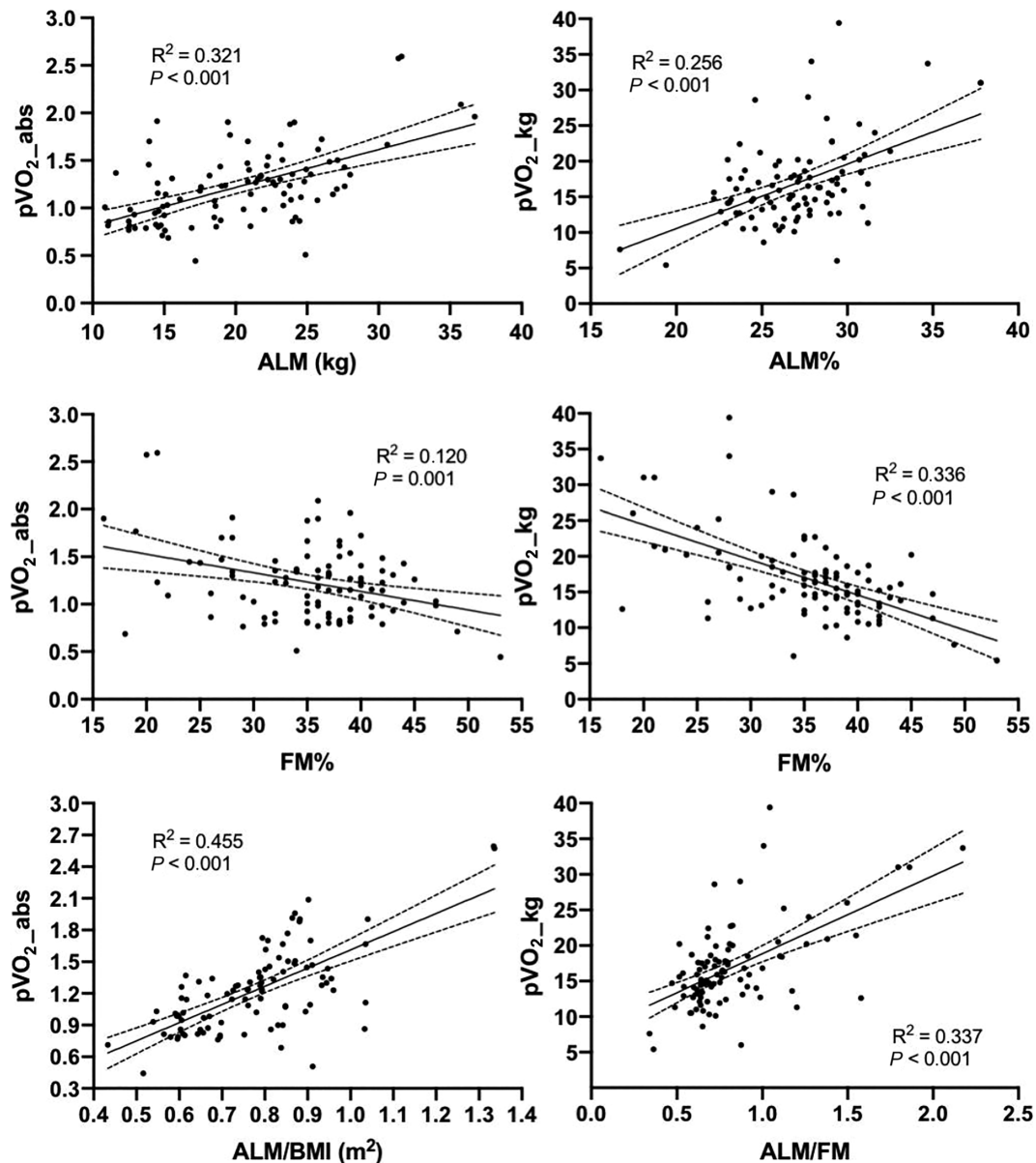
dose reduction may favour ALM gain. In fact, the cyclosporine drug dose was higher in Period 1 if compared with Period 2 in accordance with the local immunosuppression protocol. This protocol reduces daily CNI dose when HTx recipients arrive in the chronic stable phase, which is considered to begin at approximately 12 months distance to transplant operation provided that clinically relevant allograft rejection was not detected in preceding endomyocardial biopsy. In summary, these changes support biological plausibility of this inverse correlation between CNI dose and ALM.

In all periods, a substantial proportion of BC investigations was compatible with sarcopenia based exclusively on measures of ALMI or ALM/BMI below the cut-offs used in two different consensus definitions of this syndrome, which is not only characterized by low muscle mass but also reduced muscle function. Indeed, muscle strength was not assessed in the present study. Despite of a gradual increase of ALM, the proportion of ALM measures in the sarcopenic range did not significantly decrease from Periods 1 to 4. Nonetheless, 80% of ALMI measures in Period 1 stand below the 20th percentile of

Table 4b Multiple regression for pVO_2_abs with appendicular lean mass and fat mass index

R	R^2	Adj. R^2	P		
0.904	0.817[a]	0.808	<0.001		
Predictors[b]	B	95% CI for B		P	Beta
(Constant)	−0.561	Lower bound	Upper bound		
AT_abs[a] (L/min)	0.804	0.576	1.031	<0.001	0.488
ALM (kg)	0.028	0.018	0.038	<0.001	0.396
Peak HR (b.p.m.)	0.007	0.005	0.009	<0.001	0.366
FMI (kg/m²)	−0.022	−0.035	−0.008	0.001	−0.181

ALM, appendicular lean mass; B, unstandardized coefficient; Beta, standardized coefficient; CI, confidence interval; FMI, fat mass index; HR, heart rate.
[a]Missing values of AT_abs (12/95) were excluded pairwise (if excluded listwise: $R^2 = 0.827$).
[b]Gender, years after heart transplantation, age at body composition, donor age, VE/VCO₂, haemoglobin, N-terminal pro-brain natriuretic peptide, and estimated glomerular filtration rate were excluded stepwise. Only the final best fit model is illustrated.

reference values measured in a healthy Caucasian population, compared with only about 50% in Period 4.

Skeletal muscle in sarcopenic patients present with lower capillary density and capillary/fibre ratio,[27] and similar changes have been reported from HTx recipients.[28] This suggests that sarcopenia not only implies lower absolute muscle mass but also less important vascularization compatible with the lowest AT observed in Period 1, which presented the highest proportion of study participants with the lowest ALMI values. Increase of peripheral skeletal mass not only improved pVO_2 in the present study but also increased AT; therefore, improvement of vascularization is plausible but still remains to be shown.

Sarcopenia closely relates to poor physical performance and explains many manifestations of frailty, a clinical phenotype found in up to 30% of HTx recipients on waiting list based on the presence of three or more of the following features from the modified Fried Frailty Phenotype (FFP): exhaustion, reduced grip strength, reduced gait speed, reduced appetite, low physical activity, or cognitive impairment.[29] However, data limited by small sample size suggest that most recipients identified as frail before their transplant operation will recover from this state in the following post-operative months.[30] In that regard, the lowest ALMI values and exercise capacity observed at the first period post-transplant in the present study might be interpreted as stigma of frailty, and sarcopenia as a possible transition state before full recovery.

At last, FM is negatively associated with maximal exercise capacity as found by Nytrøen *et al*.[5] reporting a negative linear relationship between pVO_2_kg and body fat percentage ($b = -0.40$, $P < 0.001$) in 51 HTx recipients. In accordance with this observation, increase of peripheral muscle mass

(ALM) in parallel with increase of adiposity (whether FMI or BMI) conferred little if any advantage on pVO_2 after HTx in the present study. Furthermore, FM% was negatively correlated with pVO_2_abs ($R = -0.346$; $P = 0.001$). In addition, this effect can also explain why the significance of the correlation between ALM and CNI dose was lost if the analysis was adjusted for FM.

This negative impact of FM on pVO_2 may relate with increased muscular work resulting in more rapid exhaustion, fatty infiltration reducing muscle strength, disturbed skeletal muscle activation,[31] as well as dysfunction of the skeletal muscle cell due to proinflammation and oxidative stress related to adiposity.[32] Altogether, these observations suggest that fat reduces maximal exercise capacity after HTx not only by one principal but also multisite interaction.

Of note, impairment of the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis is a frequent finding in severe heart failure associated with lower pVO_2, ventilatory efficiency, and squelettal muscle performance.[33] Because complete resolution of this anabolic pathway has been previously reported after HTx,[34] it may be suspected to play a role in the observed changes in the present study. But to which extent GH/IGF-1 or the other anabolic hormone testosterone in men may be involved in changes of BC after HTx still remains to be defined.

In summary, our results indicate a central role of BC for pVO_2 in this middle-aged, male-predominant, and sedentary HTx recipient population with normal, or near-normal graft function. This conclusion is further substantiated by the fact that ALM/BMI explains 45% of the variance of pVO_2_abs and the finding that three BC-related parameters (ALM, FMI, and AT_abs) predicted peak VO₂ in this study population while one central haemodynamic parameter, heart rate, was predictive.

Limitations

Our study is limited by a low number of participants and the small proportion of females, but this gender distribution is nevertheless representative of the HTx population. Considering that participants' level of physical activity was not assessed with a specific questionnaire, we cannot exclude that the observed increase of peripheral muscle beyond 3 years may be partially related to the inclusion of better trained individuals, and in fact, the increase of AT_% supports this hypothesis, while the persistence of an overall low median AT_% when compared with age-matched healthy controls rather argues against it. Finally, dietary habits of participants were not evaluated, in particular the adherence to a Mediterranean diet that may offer protective effects against sarcopenia and physical disability.[35]

Conclusions

Peak oxygen consumption after HTx largely relied in this sedentary HTx recipient population with normal, or near-normal graft function on peripheral factors such as peripheral skeletal mass, adiposity, and the AT. These results suggest that maximal benefit from HTx can be obtained if lean body mass and AT increase. Our findings support continued lifestyle effort to fight adiposity and suggest that decrease of CNI dose in the chronic stable phase after transplant operation as well as rapid weaning of CS favour positive adaptation of peripheral skeletal muscle.

Conflict of interest

The authors deny any conflict of interest in relation to this work.

Funding

This work received no financial support.

Author contributions

- J.R.: study hypothesis, study design, data collection, data analysis, interpretation of the study results, and writing of the manuscript.
- P.M.: data analysis, critical revision of the manuscript, and final approval.
- P.Y., L.F., M.K., P.T., and O.L.: critical revision of the manuscript and final approval.
- R.H.: study hypothesis, study design, interpretation of the study results, and writing of the manuscript.

Supporting information

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

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Table S2. *Body composition by period after HTx.*
Table S3. *CPET findings by period after Htx.*
Table S4. *Echocardiographic findings by period after Htx.*
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Table S7. *Baseline characteristics by period in the COUPLE-dataset.*
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Table S10a. *Univariate predictors of pVO2_abs in the COUPLE-dataset.*
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Figure S1. *Study flow chart.*
Figure S2. *Paired analysis of BC in 7 participants at years 1-2-3 after HTx.*

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