Low skeletal muscle mass is associated with low aerobic capacity and increased mortality risk in patients with coronary heart disease - A CARE CR Study

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Abstract

Background: In patients with chronic heart failure, there is a positive linear relationship between skeletal muscle mass (SMM) and peak oxygen consumption ($\dot{V}O_{2peak}$); an independent predictor of all-cause mortality. We investigated the association between SMM and $\dot{V}O_{2peak}$ in patients with coronary heart disease (CHD) without a diagnosis of heart failure.

Methods: Male patients with CHD underwent maximal cardiopulmonary exercise testing and dual X-ray absorptiometry assessment. $\dot{V}O_{2peak,}$ the ventilatory anaerobic threshold and peak oxygen pulse (peak $\dot{V}O_2$/HR) were calculated. SMM was expressed as appendicular lean mass (lean mass in both arms and legs) and reported as skeletal muscle index (SMI; kg/m^2), and as a proportion of total body mass (appendicular skeletal mass [ASM%]). Low SMM was defined as a SMI<7.26 kg/m^2, or ASM% <25.72%). 5-year all-cause mortality risk was calculated using the Caliber 5-year all-cause mortality risk score.

Results: Sixty patients were assessed. Thirteen (21.7%) had low SMM. SMI and ASM% correlated positively with $\dot{V}O_{2peak}$ (r=0.431 and 0.473, respectively; P<0.001 for both). SMI and ASM% predicted 16.3% and 12.9% of the variance in $\dot{V}O_{2peak}$, respectively. SMI correlated most closely with peak $\dot{V}O_2$/HR (r=0.58; p<0.001). SMI predicted 40.3% of peak $\dot{V}O_2$/HR variance. ASM% was inversely associated with 5-year all-cause mortality risk (r=-0.365; P=0.006).

Conclusion: SMM was positively correlated with $\dot{V}O_{2peak}$ in patients with CHD. Peak $\dot{V}O_2$/HR had the strongest association with SMM. Low ASM% was associated with a higher risk of all-cause mortality. The effects of exercise and nutritional strategies aimed at improving SMM and function in CHD patients should be investigated.

Abstract Word Count: 250

Key words: Coronary Disease, Sarcopenia, Cardiorespiratory Fitness, Skeletal Muscle

Introduction

Peak oxygen uptake ($\dot{V}O_{2peak}$), measured by a maximal cardiopulmonary exercise test (CPET) represents the upper limit of aerobic capacity. A low $\dot{V}O_{2peak}$ is associated with the loss of independence in older individuals [1] and increased all-cause and cardiovascular mortality in patients with coronary heart disease (CHD] [2]. The physiological factors that limit $\dot{V}O_{2peak}$ are summarised by the Fick equation [3]:

$$\dot{V}O_{2peak} = \text{cardiac output x } (a\text{-}vO_2 \text{ diff})$$

Where *cardiac output* is the product of heart rate (HR) and stroke volume (SV), and *a-vO_2 diff* is the difference between arterial and venous O_2 content, representing muscle O_2 extraction. In healthy individuals, $\dot{V}O_{2peak}$ is limited centrally; maximum cardiac output limits O_2 delivery to the exercising muscle at the rate that it is required for aerobic resynthesis of ATP [3]. However, in patients with chronic heart failure (CHF), a cascade of events alters peripheral muscle physiology. These include, reduced skeletal muscle oxidative enzyme activity, reduced mitochondrial density, decreased perfusion matching with oxidative muscle fibres [4], and decreased skeletal muscle mass [5]. Consequently, the peripheral muscle may become the primary limitation to $\dot{V}O_{2peak}$ [6,7,4].

As CHF worsens, skeletal muscle mass [8] and $\dot{V}O_{2peak}$ declines [9,10]. However, this relationship has not yet been reported in patients with CHD. Around a quarter of patients with CHD have low muscle mass and function (sarcopenia) [11], compared with only 10% of adults older than 60 years [12]. Patients with CHD are commonly physically inactive which, together with progression of the underlying CHD, may exacerbate loss of muscle mass. Patients with CHD who experience a marked loss of skeletal muscle mass may have a reduced $\dot{V}O_{2peak}$ and consequently, higher risk of early mortality. Identifying the relationship

between skeletal muscle mass and $\dot{V}O_{2peak}$ in patients with CHD may be important so that preventative exercise and nutritional interventions can be developed.

We aimed to describe the association between skeletal muscle mass and $\dot{V}O_{2peak}$ in male patients with CHD. We also assessed the relationship between skeletal muscle mass and other potentially important variables: peak oxygen pulse (peak $\dot{V}O_2$/HR) [16], the ventilatory anaerobic threshold (VAT) [17], VE/VCO_2 slope [18], N-terminal pro B-type Natriuretic Peptide (NT-proBNP) [19] and CALIBER 5-year all-cause mortality risk [20].

Methods

Study Design

Data for this cross-sectional study included baseline measurements taken from male patients enrolled in the Cardiovascular and cardiorespiratory Adaptations to Routine Exercise-based Cardiac Rehabilitation (CARE CR) study [21]. Ethical approval was obtained from the Humber Bridge NHS Research Ethics Committee - Yorkshire and the Humber (13/YH/0278). Study procedures confirm to the declaration of Helsinki 1964.

The study protocol for CARE CR has previously been reported [21]. Briefly, clinically stable patients who had recently been discharged from hospital following an admission for stable angina, myocardial infarction (MI), coronary artery bypass graft (CABG) surgery, or elective percutaneous coronary intervention (PCI) were recruited. Patients were asked to attend the research laboratory having not participated in strenuous exercise within the previous 24 hours. Written informed consent was obtained prior to conducting any investigations.

Resting Measurements

Resting HR and left arm brachial blood pressure were taken at the end of 15 minutes semi-supine rest using a 12-lead ECG (GE Healthcare, Buckinghamshire, UK) and an ECG-gated automated blood pressure cuff (Tango, SunTech Medical, Eynsham, United Kingdom). Stature (cm) was measured using a Leicester Height Measure (SECA, Birmingham, United Kingdom). Waist circumference measurements were taken 1 cm above the iliac crest and hip measurements were taken from the widest aspect of the buttocks. Both measurements were recorded in cm and the ratio of the two was calculated to determine waist to hip circumference ratio [22].

A 2D echocardiogram was used to determine left ventricular (LV) function. LV ejection fraction (LVEF) was calculated using the Simpson's method from measurements of end-diastolic and end-systolic volumes on apical 4- chamber and 2-chamber 2D views, following the guidelines of Schiller and colleagues [23]. LV systolic dysfunction was diagnosed if LVEF was ≤45%.

Body Composition

Body composition was determined using dual X-ray absorptometry [DEXA] (Lunar iDXA, 255 GE Healthcare, Buckinghamshire, UK). Total body mass (kg), lean body mass (kg) and total fat (%) were determined using the Lunar iDXA's integrated software. BMI (kg/m^2) was calculated using DEXA-derived total body mass. Appendicular lean mass (ALM; total lean mass in both arms and legs) was calculated (kg) and indexed to derive skeletal muscle index (SMI; measured in kg/m^2). ALM was also reported as a percentage of total body mass (appendicular skeletal mass; ASM%). Low skeletal muscle mass was defined as an SMI of <7.26 kg/m^2 as recommended by international consensus guidelines [24]. A low or ASM%

was defined as <25.72 % [25]. This approach may be more appropriate for patients who are overweight/obese and have a higher absolute skeletal muscle mass, but low skeletal muscle mass relative to their total body mass

Maximal Cardiopulmonary Exercise Test

CPET adhered to established guidelines [26-29] and was performed using the modified Bruce treadmill protocol [30] (GE Healthcare, Buckinghamshire, UK). A 12-lead ECG was monitored continuously throughout the test. An ECG-gated automated BP measurement was recorded at the start of the test and at the second minute of each test stage until the end of the test. Rating of perceived exertion (RPE) scores (6-20) were recorded at peak exercise [31]. Breath-by-breath metabolic gas exchange data were collected using an Oxycon Pro metabolic cart (Jaeger, Hoechburg, Germany). $\dot{V}O_{2peak}$ was defined as the mean $\dot{V}O_2$ (ml) over the last 30 seconds of the test. $\dot{V}O_{2peak}$ was also adjusted for body mass (ml/kg/min). The VAT was analysed by two independent investigators using the V-slope method [32] with data averaged over the middle 5 of 7 consecutive breaths. The VAT was reported in ml, and standardised to patient body mass (ml/kg/min). $\dot{V}O_2$/HR (ml/beat) and $\dot{V}E/\ \dot{V}CO_2$ slope were calculated as previously described [21,29].

Pulse Wave Velocity

Pulse wave velocity (PWV) between the brachium and ankle was measured (Vascular Explorer, Enverdis GmbH, Düsseldorf, Germany) after 15 minutes of semi-supine rest (torso elevated 45°) in a quiet temperature controlled room (21°C). A blood pressure cuff was placed proximally to the left cubital fossa (brachial artery) and another placed proximally to the medial malleolus. Photoplethysmographic sensors were placed on the patients left index finger and left hallux. Oscillations in the pulse waves alter the volume of the blood pressure

cuff and are converted to a PWV. A shorter PWV (ms) indicates more severe arterial stiffness and/or worse peripheral vascular health.

Blood Samples

Resting venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA), potassium oxalate, and serum separating tubes (SST). EDTA and potassium oxalate tubes were spun in a refrigerated (4°C) centrifuge at 3,000 revolutions per minute, for 15 minutes immediately after the blood draw. Samples collected in SST tubes were allowed to clot for 30 minutes prior to being centrifuged under the same conditions. Haematocrit and haemoglobin concentrations, neutrophil and lymphocyte count, and NT-proBNP were analysed using a registered National Health Service (NHS) pathology lab (Castle Hill Hospital, Hull). All samples not analysed on the day of collection were stored in a -80°C freezer. The ABX Pentra 400 biochemistry auto analyser (Horiba, Montpellier, France) was used to analyse serum plasma glucose, and high sensitivity C-reactive protein (hs-CRP) in duplicate. Calibration and quality controls were conducted in accordance with manufacturer's guidelines.

Prognosis – Caliber 5-year all-cause mortality risk

5-year risk of all-cause mortality was calculated for each patient using the comprehensive online (https://www.caliberresearch.org/model) Caliber 5-year risk score [20]. This model does not include any fitness measurements in its calculation. 5-year risk of all-cause mortality was reported as a percentage. The variables included in the Caliber score are shown in Table 1.

Table 1 – Variables included in the CALIBER 5-year risk score

Categorical Variables	Continuous Variables
Sex	Age
Belongs to most deprived quintile	Total cholesterol
CAD diagnosis and severity	HDL
Interventions (last six months)	Heart rate
Smoking status	Creatinine
Hypertension/BP lowering medication	White cell count
Diabetes	Haemoglobin
Heart failure	
Peripheral arterial disease	
Atrial fibrillation	
Stroke	
Chronic renal disease	
COPD	
Cancer	
Chronic liver disease	
Depression	
Anxiety	

CAD = Coronary Artery Disease; BP = Blood Pressure; COPD; Chronic Obstructive Pulmonary Disease; HDL; High Density Lipoprotein

Statistical Analysis

Statistical analysis was performed using SPSS version 22 (IBM, New York, USA). Data were visually assessed for normality. Categorical data are reported as percentages. Continuous normally distributed variables are displayed as mean with 95% confidence intervals (95% CI) or standard deviation (±) where specified. Non-normally distributed data are displayed as median (range). Pearson (normally distributed), Spearman correlations (non-normally distributed) and age-adjusted partial correlations were used to assess the relationship between indices of skeletal muscle mass and variables of interest. An r value of <0.25, 0.26 to 0.50, 0.51 to 0.75, and, >0.75 were considered weak, moderate, fair and strong associations, respectively [33]. Scatter plots of partial correlations were constructed using the residuals of the independent skeletal muscle indices and dependent variables. Where a variable was significantly associated with SMI or ASM%, receiver operating characteristic (ROC) curves were used to investigate the sensitivity and specificity of predicting low skeletal muscle

mass. Patients with a low skeletal muscle mass (SMI <7.26 Kg/m^2 or ASM% <25.72%) were treated as the dichotomous 'state-variable' for the ROC curve. Statistical significance was set at P=0.05.

Up to six dependent variables with the strongest, significant age-adjusted partial correlations were selected for inclusion within separate stepwise multivariate regression models. The main outcome variables selected were $\dot{V}O_{2peak}$, VAT, peak $\dot{V}O_2$/HR, CPET duration, Caliber 5-year all-cause mortality risk and NT-proBNP.

Results

Sixty male patients (aged, 62.1 ± 10.0 years; BMI, 28.8 ± 3.7 kg/m^2) were recruited. Patient characteristics and medications are reported in Table 2 and 3, respectively. Fifteen patients (25.0%) had sustained a ST-elevation MI, 19 (31.7%) a non-ST-elevation MI, 16 (26.7%) underwent elective PCI, 6 (10.0%) CABG and 4 (6.7%) had exertional angina. Median time from cardiac event to baseline assessment was 54 days (range 22 to 220 days). Mean resting HR was 82 bpm ± 14 bpm. Mean resting systolic and diastolic blood pressure was 127 ± 17 mmHg and 58 ± 9 mmHg, respectively. Four patients (6.6%) had a LVEF <45%, ten (16.7%) had an NT-proBNP >400pg/L and one (1.7%) had an NT-proBNP >2000pg/L. The proportion of patients with low skeletal muscle mass was 16.7% by SMI, and 11.7% by ASM%. However, only 4 patients had both a low SMI *and* ASM%, meaning that 13 (21.7%) had a low SMI *or* ASM%.

Correlations between ALM, SMI, ASM% and dependent variables of interest are presented in Table 4. The associations between ALM, SMI, and $\dot{V}O_{2peak}$ (ml) were r=0.566 (p<0.001) and r=0.473 (p<0.001) respectively. The association between ASM% and $\dot{V}O_{2peak}$ (ml/kg/min) was r=0.420 (p=0.001). The strongest associations between indices of skeletal muscle mass

and secondary outcome measures were observed between; ALM and peak $\dot{V}O_2$/HR (r=0.633; p<0.001), SMI $\dot{V}O_2$/HR (r=0.575; p<0.001), and ASM% and $\dot{V}O_{2peak}$ [ml/kg/min] (r=0.431; p<0.001). CPET variables that were significantly associated with ALM, SMI and ASM% are shown in Figure 1. ASM% was the only method of characterising skeletal muscle mass resulting in a significant association with PWV, NT-proBNP and Caliber 5-year all-cause mortality risk.

Table 2 – Patient Characteristics

Variable	**Mean (± SD)**
Age (Years)	63.1 (10.0)
BMI (kg/m^2)	28.8 (3.7)
ALM (kg)	24.8 (4.0)
SMI (kg/m2)	8.3 (1.1)
ASM%	29.1 (2.5)
Body Fat%	35.5 (9.3)
Waist/Hip Ratio	0.98 (0.06)
VO_{2peak} (ml/kg/min)	24.0 (5.6)
VO_{2peak} (ml)	2079.3 (552.6)
VO_{2peak} Lean (ml/kg/min)	38.5 (8.3)
VAT (ml/kg/min)	17.2 (5.1)
VAT (ml)	1481.9 (452.4)
VAT Lean (ml/kg/min)	27.8 (7.6)
Peak O_2/HR (ml/beat)	15.5 (3.3)
Peak HR	134 (20)
VE/VCO_2 Slope	34.3 (6.0)
CPET Duration (seconds)	826.6 (193.8)
LVEF (%)	54.3 (6.7)
Caliber 5 Year Risk (%)	8.3 (7.0)
NT-proBNP (pg/L)	174.5 (11.4 to 2735.0)
hs-CRP (mg/L	2.3 (3.0)
Glucose (mmol)	6.1 (2.0)

BMI = Body Mass Index; ALM = Appendicular Lean Mass; SMI = Skeletal Muscle Index; ASM% = Appendicular Skeletal Mass; VO_{2peak} = Peak Oxygen Uptake; VAT = Ventilatory Anaerobic Threshold; O_2/HR = Oxygen Pulse; VE/VCO_2 slope = Ventilatory Efficiency with Respect to Carbon Dioxide Elimination; CPET = Cardiopulmonary Exercise Test; LVEF = Left Ventricular Ejection Fraction; NT-proBNP = N-Terminal pro B-type Natriuretic Peptide; hs = High Sensitivity

Table 3 – Patient Medications

Medications	**All Patients**
Aspirin (%)	58 (96.7)
Clopidogrel (%)	16 (26.7)
Ticagrelor (%)	33 (55.0)
Beta-Blockers (%)	54 (90.0)
ACE Inhibitors (%)	38 (63.3)
Statins (%)	57 (95.0)
Diuretics (%)	5 (8.3)
Nitrates (%)	13 (21.7)
GTN (%)	54 (90.0)

ACE = Angiotensin Converting Enzyme;
GTN = Glyceryl Trinitrate

ROC Curve Analysis

ROC curve analysis was conducted on variables that were significantly associated with measurements of SMI or ASM%. The area under the curve (AUC) for each prognostic variable is shown in Table 5. For SMI, peak $\dot{V}O_2$/HR had the greatest predictive (AUC = 0.767; p=0.008). Values <13.3 ml/beat was predictive of a low SMI. When patients with a low SMI or ASM% were combined, peak $\dot{V}O_2$/HR AUC was 0.764 (p=0.004). A peak $\dot{V}O_2$/HR <14.3 ml/beat was most predictive of patients with a low SMI or ASM%. Patients with a NT-proBNP >112.5 pg/L, a Caliber risk score >3.0% or a modified Bruce treadmill duration <17 Minutes 43 seconds were also more likely to have a low ASM%.

Multivariate Regression

ALM and age (both p<0.001) were independent predictors of $\dot{V}O_{2peak}$ (ml) and VAT (ml). For $\dot{V}O_{2peak}$, these variables explained 58.8% of variance. For VAT 34.8% was explained. ALM alone accounted for 43.4% and 23.9% of variance for $\dot{V}O_{2peak}$ and VAT, respectively. ALM was the only significant predictor of peak $\dot{V}O_2$/HR (p<0.001), accounting for 49.8% of variance.

Table 4 – Correlation and Partial Correlations between appendicular lean mass, skeletal muscle index, appendicular skeletal mass and dependent variables

Variable	Appendicular Lean Mass				Skeletal Muscle Index				Appendicular Skeletal Mass			
	Pearson Correlation (r)	***p*-value**	Partial Correlation (r)	***p*-value**	Pearson Correlation (r)	***p*-value**	Partial Correlation (r)	***p*-value**	Pearson Correlation (r)	***p*-value**	Partial Correlation (r)	***p*-value**
VO_{2peak} (ml)	0.666	0.001*	0.566	<0.001*	0.577	<0.001*	0.473	<0.001*	0.225	0.084	0.205	0.130
VO_{2peak} (ml/kg/min)	0.189	0.148	-0.007	0.962	0.196	0.134	0.042	0.758	0.420	<0.001*	0.431	0.001*
VAT (ml)	0.502	0.001*	0.360	0.006*	0.496	<0.001*	0.365	0.006*	0.167	0.203	0.148	0.277
VAT (ml/kg/min)	0.053	0.686	-0.092	0.502	0.127	0.333	0.009	0.946	0.310	0.016*	0.312	0.019*
Peak O_2/HR (ml/beat)	0.711	0.001*	0.633	<0.001*	0.643	<0.001*	0.575	<0.001*	0.202	0.121	0.163	0.230
Peak HR	0.165	0.208	-0.042	0.752	0.110	0.402	-0.073	0.581	0.076	0.565	0.026	0.845
VE/VCO_2 Slope	-0.272	0.036*	-0.078	0.566	-0.270	0.037*	-0.099	0.468	-0.195	0.135	-0.158	0.245
CPET Duration (seconds)	0.182	0.165	0.014	0.920	0.206	0.114	-0.047	0.730	0.376	0.003*	0.399	0.022*
LVEF (%)	0.153	0.244	0.077	0.574	0.137	0.297	0.068	0.620	0.076	0.566	0.055	0.698
Caliber 5 Year Risk (%)	-0.426	0.001*	-0.230	0.870	-0.372	0.003*	-0.224	0.098	-0.330	0.010*	-0.365	0.006*
NT-proBNP (pg/L)[a]	-0.295	0.025*	0.155	0.254	-0.253	0.056	-0.131	0.337	-0.331	0.011*	-0.326	0.014*
hs-CRP(mg/L)	-0.092	0.486	-0.101	0.458	0.000	0.999	-0.004	0.974	-0.193	0.140	-0.184	0.175
Glucose (mmol)	0.036	0.789	-0.051	0.708	0.135	0.314	0.065	0.634	-0.223	0.093	-0.242	0.073
Body Fat (%)	0.149	0.254	0.153	0.261	0.151	0.249	0.148	0.275	-0.401	0.001*	-0.406	0.002*

VO_{2peak} = Peak Oxygen Uptake; VAT = Ventilatory Anaerobic Threshold; O_2/HR = Oxygen Pulse; HR = Heart Rate; VE/VCO_2 slope = Ventilatory Efficiency with Respect to Carbon Dioxide Elimination; CPET = Cardiopulmonary Exercise Test; LVEF = Left Ventricular Ejection Fraction; NT-proBNP = N-Terminal pro B-type Natriuretic Peptide; hs = High Sensitivity

*=Significant; [a] = Spearman Correlation

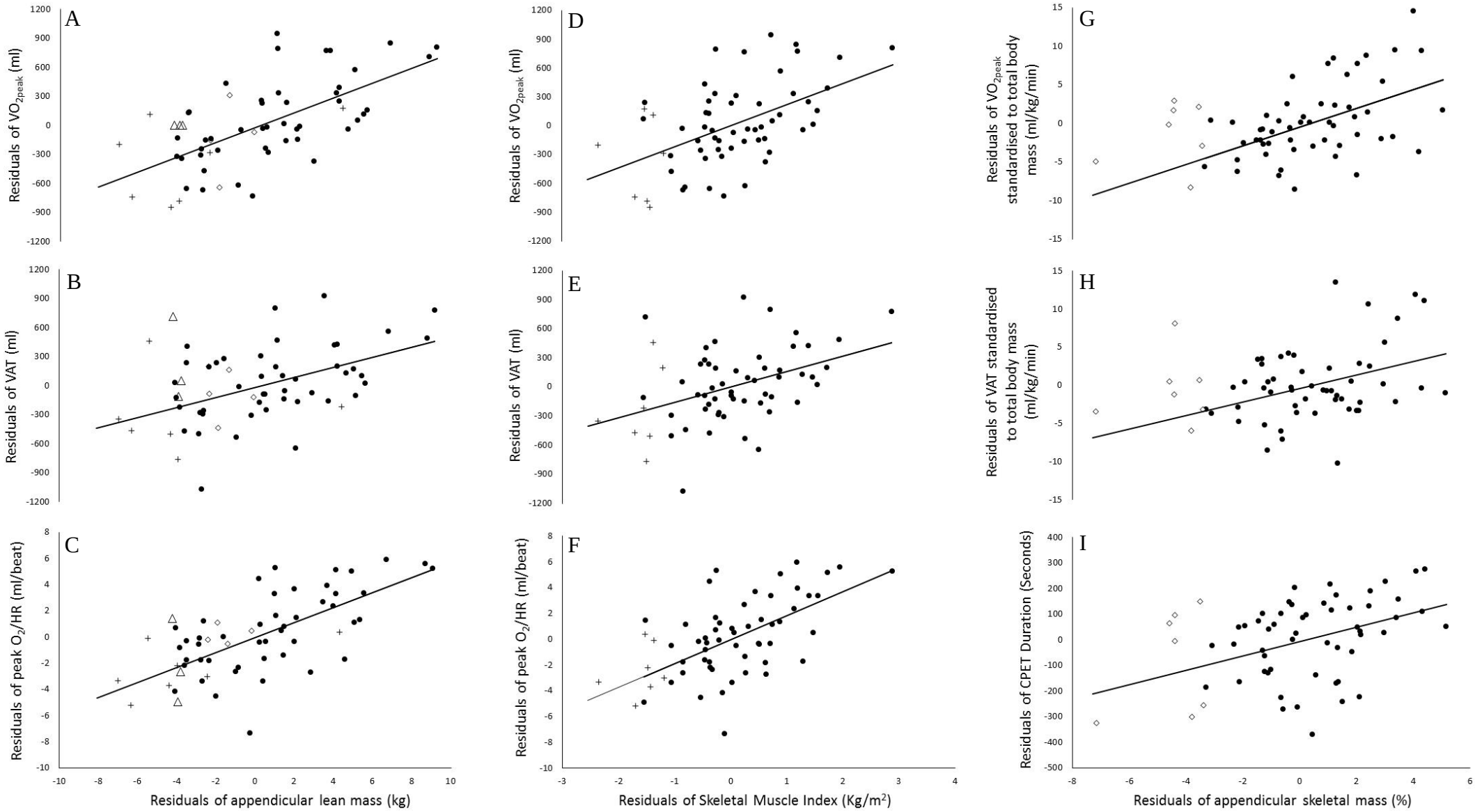

Figure 1 - Partial correlations between appendicular lean mass and VO_{2peak} (A), VAT (B), and peak VO_2/HR (C). Panels D to F show partial correlations between skeletal muscle index and VO_{2peak} (D), VAT (E), and peak O_2/HR (F). Panels G to H show partial correlations between appendicular skeletal mass and VO_{2peak} (G), VAT (H), and total CPET Duration (I)

VO_{2peak} = Peak Oxygen Uptake; VAT = Ventilatory Anaerobic Threshold; VO_2/HR = Oxygen Pulse; CPET = Cardiopulmonary Exercise Test
*=Significant; + = Low Muscle Mass When Defined Using Skeletal Muscle Index (<7.26Kg/m^2); ◊ = Low Muscle Mass When Defined Using Appendicular Skeletal Mass (<25.72%); Δ = Low Muscle Mass When Using Either Skeletal Mass or Appendicular Skeletal Mass

SMI and age were independent predictors of $\dot{V}O_{2peak}$ and the VAT (ml). For $\dot{V}O_{2peak}$, 52.5% of variance was explained by SMI and age, with SMI accounting for 16.3%. For VAT, 35.8% of variance was explained by SMI and age. 12.1% of variance was explained by VAT alone. Interestingly, only SMI was an independent predictor for peak $\dot{V}O_2$/HR. 40.3% of variance was accounted for by $\dot{V}O_2$/HR.

Similar to SMI, ASM% and age (both $p<0.001$) were independent predictors of $\dot{V}O_{2peak}$ and VAT, standardised to body mass (ml/kg/min). 38.8% of $\dot{V}O_{2peak}$ (ml/kg/min) and 17.8% of VAT (ml/kg/min) variance was accounted for by ASM% and age. ASM% alone accounted for 12.9% of $\dot{V}O_{2peak}$ variance, compared to 6.3% for VAT. Age and ASM% were also independent predictors of NT-proBNP ($p<0.001$) with age accounting for 20.5% of NT-proBNP variance and ASM% accounting for 7.2% (combined model; 27.2%). ASM% accounted for 9.4% of total Caliber 5-year all-cause mortality risk score variance ($p<0.001$).

Discussion

To our knowledge, this is the first study to investigate the relationship between skeletal muscle mass and $\dot{V}O_{2peak}$ in patients with CHD. We identified that a lower skeletal muscle mass was associated with a lower $\dot{V}O_{2peak}$, an observation previously reported in patients with CHF [5]. We also investigated the relationship between other important prognostic indicators, including peak $\dot{V}O_2$/HR. Our data suggests that skeletal muscle mass may be more closely associated with peak $\dot{V}O_2$/HR, rather than $\dot{V}O_{2peak}$. Similar to previously reported data in patients with CHD [11], we found that more than one-fifth of patients had a low skeletal muscle mass; a higher proportion than reported among adults over the age of 60 years (10%) [12]. A lower skeletal muscle mass was associated with a higher 5-year all-cause mortality risk.

Table 5 – Area under the curve for variables associated low skeletal muscle mass

Variable	Skeletal Muscle Index		Appendicular Skeletal Mass		Skeletal Muscle Index & Appendicular Skeletal Mass	
	Area Under the Curve	*p*-value	Area Under the Curve	*p*-value	Area Under the Curve	*p*-value
VO_{2peak} (mL)	0.649 (0.478 to 0.820)	0.139	-	-	0.675 (0.527 to 0.823)	0.055
VO_{2peak} (mL/kg/min)	-	-	0.726 (0.510 to 0.943)	0.053	0.615 (0.430 to 0.810)	0.206
Peak O_2/HR (mL/beat)	0.767 (0.613 to 0.921)	0.008*	-	-	0.764 (0.632 to 0.895)	0.004*
VAT (mL)	0.594 (0.399 to 0.789)	0.351	-	-	0.620 (0.455 to 0.786)	0.187
VAT (mL/kg/min)	-		0.679 (0.454 to 0.904)	0.126	0.561 (0.368 to 0.755)	0.501
CPET Duration (Seconds)	-		0.744 (0.510 to 0.943)	0.037*	0.648 (0.466 to 0.830)	0.104
Caliber 5-Year Risk (%)	-		0.805 (0.600 to 1.00)	0.009*	0.383 (0.191 to 0.574	0.202
NT-proBNP (pg/L)	-		0.759 (0.511 to 1.00)	0.027*	0.623 (0.420 to 0.825)	0.178

VO_{2peak} = Peak Oxygen Uptake; O_2/HR = Oxygen Pulse; VAT = Ventilatory Anaerobic Threshold; CPET = Cardiopulmonary Exercise Test; N-Terminal pro B-type Natriuretic Peptide;

*=Significant

Peak Oxygen Uptake

Sarcopenia and/or a low muscle mass are associated with increased mortality risk [34] and difficulties performing daily activities [35]. A low $\dot{V}O_{2peak}$ is also associated with a higher mortality risk [2] and difficulties performing daily activities [1]. Consistent with data reporting on patients with CHF (r=0.46 to r=0.70) [10,5], we found that ALM was positively associated with $\dot{V}O_{2peak}$ (r=0.566). Although dependent on the methods used to scale $\dot{V}O_{2peak}$, this relationship was maintained when ALM was standardised to stature (SMI; r=0.473) and body mass (ASM%; r=431). However, whilst ALM (43.4%), SMI (16.3%) and ASM% (12.9%) were independent predictors of $\dot{V}O_{2peak}$, we were unable to replicate the same predictive strength (54-65%) reported by Cicoira et al. [5] in non-cachexic patients with CHF. This may be because patients with CHF can have severe skeletal muscle abnormalities [36,7,4,37] that limit exercise tolerance as a consequence of the disease [6]. Given that CHD represents an earlier stage of cardiovascular dysfunction than CHF, skeletal muscle abnormalities may occur at an earlier stage of cardiovascular dysfunction. Skeletal muscle mass may therefore play a greater role in limiting $\dot{V}O_{2peak}$ among patients with CHF, compared with patients who have CHD. Our observation may suggest that adverse changes in skeletal muscle mass and quality associated with cardiovascular dysfunction exist on a continuum. If this were true, optimisation strategies that target peripheral muscle in addition to cardiac function may be important for disease prevention.

Peak $\dot{V}O_2$/HR was most closely associated with indices of skeletal muscle mass. ALM and SMI separately accounted for 49.8% and 40.3% of the variance in peak $\dot{V}O_2$/HR, respectively. These indices of skeletal muscle mass not only had a stronger association with peak $\dot{V}O_2$/HR (r=0.575 to r=0.633) compared to $\dot{V}O_{2peak}$ (r=0.566), but also had the largest AUC (0.767; 95% CI 0.613 to 0.921; p=0.008). Unlike $\dot{V}O_{2peak,}$ peak $\dot{V}O_2$/HR had good

sensitivity and specificity for detecting patients with a low SMI. A threshold of 13.3 ml/beat was identified as the point below which, patients were more likely to have a low SMI (7.26 kg/m^2). Simple rearrangement of the Fick equation means that peak $\dot{V}O_2$/HR becomes independent of HR; a factor that we (r=-0.042, -0.073, 0.026; p>0.05 for all) and others [13,14] have found to be unrelated to skeletal muscle. Instead peak $\dot{V}O_2$/HR characterises SV and a-vO_2 difference (peripheral O_2 extraction) which may explain why it appears to be more closely related to skeletal muscle mass than $\dot{V}O_{2peak}$, in patients with CHD. However, whilst skeletal muscle mass has previously been identified as an independent predictor of peak $\dot{V}O_2$/HR (74%) in hypertensive men and women [15], the association between peak indices of skeletal muscle mass and peak $\dot{V}O_2$/HR in our study was smaller [40.3 to 49.8%] [15]. Nonetheless, this is an interesting finding and should be further explored in a larger cohort.

The relationship between indices of skeletal muscle mass and peak $\dot{V}O_2$/HR observed in our study may reflect the inclusion of SV into the equation for peak $\dot{V}O_2$/HR and, the physiological differences between patients with hypertension [15] and patients with CHD. Under normal circumstances, $\dot{V}O_2$/HR rises progressively throughout an incremental exercise test until it reaches a plateau associated with normal physiological limitation to exercise [38]. However, in patients with CHD, $\dot{V}O_2$/HR may prematurely decrease during incremental exercise due to ischaemia-induced myocardial wall-motion abnormalities which cause a reduction in SV [39]. This is thought to occur prior to ST-segment changes detected using electrocardiogram, or symptoms of angina [40]. If myocardial blood flow is not restored through surgical or medical intervention, $\dot{V}O_2$/HR would be lower than expected at peak exercise, and may attenuate the association between $\dot{V}O_2$/HR, a-vO_2 difference and skeletal muscle mass. Alternatively, a low peak $\dot{V}O_2$/HR may indicate greater cardiac dysfunction and consequently, more severe skeletal muscle abnormalities. However, whilst we cannot

confirm either of these scenarios, peak $\dot{V}O_2$/HR was a better predictor of SMI than any other measured variable, including $\dot{V}O_{2peak,}$ the gold-standard measurement of aerobic exercise capacity. Formal screening for sarcopenia in patients who are incidentally found to have a low peak $\dot{V}O_2$/HR may be beneficial.

Prognostically Important Associations with Skeletal Muscle Mass

Although SMI was associated with peak $\dot{V}O_2$/HR, ASM% was not. However, ASM% was inversely associated with prognostically important variables including; $\dot{V}O_{2peak,}$ PWV, 5-year all-cause mortality risk, a higher NT-proBNP and, body fat percentage even when controlling for age. Although not specifically investigated by our study, the faster PWV speeds observed among patients with a lower SMI indicates more severe arterial stiffness and greater abnormalities in peripheral cardiovascular health, something reported among patients with CHF [4]. Furthermore, the higher NT-proBNP values reported among patients with a lower ASM% suggests more advanced CHD, or the early development of CHF which may exacerbate the loss of skeletal muscle mass [8]. The association between higher body fat percentage and relative lower skeletal muscle mass may also indicate the onset of sarcopenic obesity, fat infiltration of skeletal muscle and therefore, reduced muscle quality [41].

Strengths and Weaknesses

This is the first study to use gold-standard measurement techniques (DXA and CPET) to investigate the relationship between low skeletal muscle mass and reduced aerobic exercise capacity in CHD patients. In addition, this study included a representative cohort of patients with CHD and the statistical methods employed controlled for several variables that are commonly associated with reduced skeletal muscle mass and aerobic exercise capacity. However, this study has limitations; first, we did not assess muscle function, which meant

that we could not report the prevalence of sarcopenia in our cohort. Second, our findings in our male only cohort may not be relevant to females with CHD. Finally, whilst the associations identified in our cross-sectional cohort study are interesting, a prospective long-term follow up study is required. This would help to determine whether progression of CHD confers with reduced skeletal muscle mass and whether this relates to prognostically important variables and/or the development of CHF.

Conclusion

We found a high incidence of low skeletal muscle mass in our cohort of patients with CHD. When standardised to body mass (ASM%), low skeletal muscle mass conferred a higher predicted risk of all-cause mortality. Low skeletal muscle mass was associated with a low $\dot{V}O_{2peak}$ in patients with CHD, however the relationship was complex and dependent on the method used to scale skeletal muscle mass and $\dot{V}O_{2peak}$. Interestingly, our data shows that there was a stronger association between SMI and peak $\dot{V}O_2$/HR. The relationship between low skeletal muscle mass, prognostic indices and aerobic fitness suggests that adverse changes in skeletal muscle mass may be initiated before the diagnosis or development of CHF. These findings may highlight a need for preventative exercise and nutritional strategies to improve skeletal muscle mass and quality in patients with CHD.

Author Contributions

SN – Contributed to project conception and design, acquisition, analysis and interpretation of data, drafted and critically revised the manuscript.

AFO'D - Contributed to analysis and interpretation of data, and drafted and critically revised the manuscript.

CT- Contributed to the acquisition of data and critically revised the manuscript.

ALC - Contributed to project design and critically revised the manuscript

SC and LI - Contributed to project conception and design, analysis and interpretation of data, drafted and critically revised the manuscript.

All authors have given their final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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References

1. Shephard RJ (2009) Maximal oxygen intake and independence in old age. British Journal of Sports Medicine 43 (5):342-346. doi:10.1136/bjsm.2007.044800
2. Keteyian SJ, Brawner CA, Savage PD, Ehrman JK, Schairer J, Divine G, Aldred H, Ophaug K, Ades PA (2008) Peak aerobic capacity predicts prognosis in patients with coronary heart disease. Am Heart J 156 (2):292-300
3. Bassett D, Howley E (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance. Medicine and Science in Sports and Exercise 32 (1):70-84
4. Poole DC, Hirai DM, Copp SW, Musch TI (2012) Muscle oxygen transport and utilization in heart failure: implications for exercise (in) tolerance. American Journal of Physiology-Heart and Circulatory Physiology 302 (5):H1050-H1063
5. Cicoira M, Zanolla L, Franceschini L, Rossi A, Golia G, Zamboni M, Tosoni P, Zardini P (2001) Skeletal muscle mass independently predicts peak oxygen consumption and ventilatory response during exercise in noncachectic patients with chronic heart failure. Journal of the American College of Cardiology 37 (8):2080-2085. doi:https://doi.org/10.1016/S0735-1097(01)01306-7
6. Shelton RJ, Ingle L, Rigby AS, Witte KK, Cleland JG, Clark AL (2010) Cardiac output does not limit submaximal exercise capacity in patients with chronic heart failure. European journal of heart failure 12 (9):983-989
7. Clark AL, Poole-Wilson PA, Coats AJS (1996) Exercise limitation in chronic heart failure: Central role of the periphery. Journal of the American College of Cardiology 28 (5):1092-1102. doi:10.1016/S0735-1097(96)00323-3
8. Collamati A, Marzetti E, Calvani R, Tosato M, D'Angelo E, Sisto AN, Landi F (2016) Sarcopenia in heart failure: mechanisms and therapeutic strategies. Journal of Geriatric Cardiology : JGC 13 (7):615-624. doi:10.11909/j.issn.1671-5411.2016.07.004
9. Fülster S, Tacke M, Sandek A, Ebner N, Tschöpe C, Doehner W, Anker SD, Von Haehling S (2013) Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF). European heart journal 34 (7):512-519
10. Piepoli MF, Kaczmarek A, Francis DP, Davies LC, Rauchhaus M, Jankowska EA, Anker SD, Capucci A, Banasiak W, Ponikowski P (2006) Reduced Peripheral Skeletal Muscle Mass and Abnormal Reflex Physiology in Chronic Heart Failure. Circulation 114 (2):126-134. doi:10.1161/circulationaha.105.605980
11. Harada H, Kai H, Niiyama H, Nishiyama Y, Katoh A, Yoshida N, Fukumoto Y, Ikeda H (2016) Effectiveness of cardiac rehabilitation for prevention and treatment of sarcopenia in patients with cardiovascular disease-A retrospective cross-sectional analysis. The journal of nutrition, health & aging:1-8
12. Shafiee G, Keshtkar A, Soltani A, Ahadi Z, Larijani B, Heshmat R (2017) Prevalence of sarcopenia in the world: a systematic review and meta- analysis of general population studies. Journal of Diabetes and Metabolic Disorders 16:21. doi:10.1186/s40200-017-0302-x
13. Piepoli MF, Corrà U, Agostoni P (2017) Cardiopulmonary Exercise Testing in Patients with Heart Failure with Specific Comorbidities. Annals of the American Thoracic Society 14 (Supplement 1):S110-S115

14. Horwich TB, Leifer ES, Brawner CA, Fitz-Gerald MB, Fonarow GC (2009) The Relationship Between Body Mass Index and Cardiopulmonary Exercise Testing in Chronic Systolic Heart Failure. Am Heart J 158 (4 Suppl):S31-S36. doi:10.1016/j.ahj.2009.07.016
15. Lim JG, McAveney TJ, Fleg JL, Shapiro EP, Turner KL, Bacher AC, Ouyang P, Stewart KJ (2005) Oxygen pulse during exercise is related to resting systolic and diastolic left ventricular function in older persons with mild hypertension. Am Heart J 150 (5):941-946. doi:https://doi.org/10.1016/j.ahj.2004.12.021
16. Laukkanen JA, Kurl S, Salonen JT, Lakka TA, Rauramaa R (2006) Peak oxygen pulse during exercise as a predictor for coronary heart disease and all cause death. Heart 92 (9):1219-1224
17. Gitt AK, Wasserman K, Kilkowski C, Kleemann T, Kilkowski A, Bangert M, Schneider S, Schwarz A, Senges J (2002) Exercise Anaerobic Threshold and Ventilatory Efficiency Identify Heart Failure Patients for High Risk of Early Death. Circulation 106 (24):3079-3084. doi:10.1161/01.cir.0000041428.99427.06
18. Van de Veire NR, Van Laethem C, Philippé J, De Winter O, De Backer G, Vanderheyden M, De Sutter J (2006) VE/Vco2 slope and oxygen uptake efficiency slope in patients with coronary artery disease and intermediate peakVo2. European Journal of Cardiovascular Prevention & Rehabilitation 13 (6):916-923. doi:10.1097/01.hjr.0000238400.35094.72
19. Omland T, Sabatine MS, Jablonski KA, Rice MM, Hsia J, Wergeland R, Landaas S, Rouleau JL, Domanski MJ, Hall C, Pfeffer MA, Braunwald E (2007) Prognostic value of B-Type natriuretic peptides in patients with stable coronary artery disease: the PEACE Trial. J Am Coll Cardiol 50 (3):205-214. doi:10.1016/j.jacc.2007.03.038
20. Rapsomaniki E, Shah A, Perel P, Denaxas S, George J, Nicholas O, Udumyan R, Feder GS, Hingorani AD, Timmis A, Smeeth L, Hemingway H (2014) Prognostic models for stable coronary artery disease based on electronic health record cohort of 102 023 patients. Eur Heart J 35 (13):844-852. doi:10.1093/eurheartj/eht533
21. Nichols S, Nation F, Goodman T, Clark AL, Carroll S, Ingle L (2018) CARE CR-Cardiovascular and cardiorespiratory Adaptations to Routine Exercise-based Cardiac Rehabilitation: a study protocol for a community-based controlled study with criterion methods. BMJ Open 8 (1). doi:10.1136/bmjopen-2017-019216
22. ACSM (2013) ACSM's Guidelines for exercise testing and prescription. 9th edn. Wolters Kluwer/Lippincott Williams & Wilkins Health, Philadelphia
23. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, et al. (1989) Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 2 (5):358-367
24. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel J-P, Rolland Y, Schneider SM (2010) Sarcopenia: European consensus on definition and diagnosis Report of the European Working Group on Sarcopenia in Older People. Age and ageing 39 (4):412-423
25. Levine ME, Crimmins EM (2012) The impact of insulin resistance and inflammation on the association between sarcopenic obesity and physical functioning. Obesity (Silver Spring, Md) 20 (10):2101-2106. doi:10.1038/oby.2012.20
26. American Thoracic Society/American College of Chest Physicians (2003) ATS/ACCP Statement on cardiopulmonary exercise testing. American Journal of Respiratory and Critical Care Medicine 167 (2):211. doi:10.1164/rccm.167.2.211

27. Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ, Lavie CJ, Macko R, Mancini D, Milani RV (2010) Clinician's Guide to Cardiopulmonary Exercise Testing in Adults: A Scientific Statement From the American Heart Association. Circulation 122 (2):191-225. doi:10.1161/CIR.0b013e3181e52e69
28. Taylor C, Nichols S, Ingle L (2015) A clinician's guide to cardiopulmonary exercise testing 1: an introduction. British Journal of Hospital Medicine 76 (4):192-195. doi:10.12968/hmed.2015.76.4.192
29. Nichols S, Taylor C, Ingle L (2015) A clinician's guide to cardiopulmonary exercise testing 2: test interpretation. British Journal of Hospital Medicine 76 (5):281-289. doi:doi:10.12968/hmed.2015.76.5.281
30. Bruce RA, Kusumi F, Hosmer D (1973) Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. Am Heart J 85 (4):546-562. doi:http://dx.doi.org/10.1016/0002-8703(73)90502-4
31. Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14 (5):377-381
32. Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60 (6):2020-2027
33. Berg KE, Latin RW (2008) Essentials of research methods in health, physical education, exercise science, and recreation. Lippincott Williams & Wilkins,
34. Cawthon PM, Marshall LM, Michael Y, Dam TT, Ensrud KE, Barrett-Connor E, Orwoll ES (2007) Frailty in older men: prevalence, progression, and relationship with mortality. J Am Geriatr Soc 55 (8):1216-1223. doi:10.1111/j.1532-5415.2007.01259.x
35. Janssen I (2006) Influence of sarcopenia on the development of physical disability: the Cardiovascular Health Study. Journal of the American Geriatrics Society 54 (1):56-62
36. Piepoli MF, Guazzi M, Boriani G, Cicoira M, Corrá U, Libera LD, Emdin M, Mele D, Passino C, Vescovo G, Vigorito C, Villani G, Agostoni P, Working Group 'Exercise Physiology SC, Cardiology CRotISo (2010) Exercise intolerance in chronic heart failure: mechanisms and therapies. Part II. European Journal of Cardiovascular Prevention & Rehabilitation 17 (6):643-648. doi:10.1097/HJR.0b013e32833f3aa5
37. Vescovo G, Serafini F, Facchin L, Tenderini P, Carraro U, Dalla Libera L, Catani C, Ambrosio G (1996) Specific changes in skeletal muscle myosin heavy chain composition in cardiac failure: differences compared with disuse atrophy as assessed on microbiopsies by high resolution electrophoresis. Heart 76 (4):337-343
38. Whipp BJ, Higgenbotham MB, Cobb FC (1996) Estimating exercise stroke volume from asymptotic oxygen pulse in humans. Journal of Applied Physiology 81 (6):2674-2679
39. Belardinelli R, Lacalaprice F, Carle F, Minnucci A, Cianci G, Perna G, D'Eusanio G (2003) Exercise-induced myocardial ischaemia detected by cardiopulmonary exercise testing. Eur Heart J 24 (14):1304-1313. doi:10.1016/S0195-668X(03)00210-0
40. Nesto RW, Kowalchuk GJ (1987) The ischemic cascade: Temporal sequence of hemodynamic, electrocardiographic and symptomatic expressions of ischemia. The American Journal of Cardiology 59 (7):C23-C30. doi:http://dx.doi.org/10.1016/0002-9149(87)90192-5
41. Narici MV, Maffulli N (2010) Sarcopenia: characteristics, mechanisms and functional significance. British medical bulletin 95:139-159. doi:10.1093/bmb/ldq008