

Supplemental Material

Data S1.

Supplemental Methods

Study population

The Study of Health in Pomerania (SHIP)

Briefly, between 1997 and 2001 a random cluster sample of 6,265 subjects (aged 20 to 79) was drawn from the population of West Pomerania, a region in the North east of Germany. A total of 4,308 (2,193 women) of them participated in the baseline (SHIP-0) study (response = 68.8%). In the first examination follow-up (SHIP-1), which was realized from 2002 to 2006, of 3,949 eligible persons, 3,300 subjects were reexamined (follow-up response = 83.6%). In the second examination follow-up (SHIP-2), which took place between 2008 and 2012, of 3,708 eligible persons, 2,333 subjects were reexamined (follow-up response = 62.9%)²⁴. Between 2008 and 2012, while SHIP-2 was being conducted, a second independent cohort was established, called SHIP-TREND, covering a population from the same region as SHIP. A stratified random sample of 8,826 adults (aged 20 to 79) was selected. Subjects, who participated in the initial SHIP cohort, were excluded from SHIP-TREND. Thus, 4,420 individuals were examined in SHIP-TREND (response = 50,1%)²⁴.

Interview, medical and laboratory examination

Trained and certified medical staff used standardized computer-assisted interviews to ask the participants about their age, sex, smoking habits (current smoker, nonsmoker or former smoker), physical activity behavior and alcohol consumption. Physical inactivity was defined as less than one hour per week of leisure time exercise, during summer or winter. Assessment of alcohol consumption

(in grams of ethanol per day) was based on data regarding consumption of beer, wine, and spirits during the last 30 days²⁴. History of myocardial infarction or stroke was self-reported.

All participants underwent an extensive standardized medical examination. Anthropometric measurements included height and weight based on recommendations of the World Health Organization (WHO)⁵⁵. Weight was measured to the nearest 0.1 kg in light clothing and without shoes using standard digital scales. BMI was calculated as weight (kg) / height² (m²). Waist circumference (WC) was assessed to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane. The subjects were standing comfortably with body weight evenly distributed between both feet. Waist-to-hip ratio was calculated as WC divided by height. FFM and fat mass (FM) were measured by bioelectrical impedance analysis (BIA) using a multifrequency Nutriguard-M device (Data Input, Pöcking, Germany) and the NUTRI4 software (Data Input, Pöcking, Germany) in participants without pacemakers. BP was assessed after a five-minute resting period in sitting position. Systolic and diastolic BP as well as heart rate were measured three times, with three minutes rest in between, on the right arm using a digital BP monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). The mean of the second and third reading was used for the present analyses. Mean arterial pressure was calculated as $(2/3) \times \text{diastolic BP} + (1/3) \times \text{systolic BP}$. Antihypertensive medication was defined as use of agents with the anatomic, therapeutic, and chemical (ATC) code C02, C03, C07, C08 and C09. Hypertensive patients were identified by either self-reported antihypertensive medication or a systolic BP above 140 mmHg and/or a diastolic value more than 90 mmHg. Fasting (defined as at least 8 hours since the last meal) and non-fasting venous blood samples were obtained from all study participants in supine position between 7 am

and 4 pm. Diabetes mellitus was defined as self-reported and/or glycated hemoglobin $\geq 6.5\%$ and/or non-fasting glucose ≥ 11.1 mmol/l and/or current self-reported use of any hypoglycemic medication defined by the ATC code A10.

Serum levels of total cholesterol, low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), and triglycerides were assessed photometrically (Hitachi 704, Roche, Mannheim, Germany). Hypercholesterolemia was defined as serum cholesterol ≥ 6.2 mmol/l and/or LDLC ≥ 4.1 mmol/l and/or total cholesterol/HDLC ratio ≥ 5.0 and /or self-reported use of any lipid-lowering medication defined by the ATC code C10. The eGFR was determined according to the Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) equation ⁵⁶ and expressed in ml/min/1.73 m²:

$$\text{eGFR} = 141 \times \min(\text{serum creatinine}/\kappa)^\alpha \times \max(\text{serum creatinine}/\kappa) - 1.209 \times 0.993^{\text{age}} \times 1.018 \text{ (if female)}$$
, where κ is 0.7 for female and 0.9 for male, α is -0.329 for female and -0.411 for male, min indicates the minimum of serum creatinine/ κ or 1, and max indicates the maximum of serum creatinine / κ or 1.

Exercise testing and gas exchange variables

A symptom-limited CPET using a calibrated electromagnetically braked cycle ergometer (Ergoselect 100, Ergoline, Germany) was performed with a physician in attendance according to a modified Jones protocol (3 min of rest, 1 min of unloaded cycling at 60 rpm, 1 min increases in work load of 16 W/min until symptom-limited (volitional exertion, dyspnea or fatigue) or terminated by the physician because of chest pain or ECG abnormalities, and 5 min of recovery) ^{26,27}. All tests were performed at room air according to current guidelines for exercise testing, with continuous monitoring of electrocardiogram, blood pressure and pulse oximetry. Gas exchange and ventilatory variables $\text{VO}_{2\text{peak}}$ was analyzed breath by breath averaged

over 10-second intervals using a computer-based system called VIASYS HEALTHCARE system (Oxycon Pro, Rudolph's mask, JAEGER/VIASYS Healthcare system; Hoechberg, Germany) ²⁶. Exercise duration was defined from the start of exercise (without resting period) up to its termination. VO_{2peak} in l/min was defined as the highest 10-second average of absolute oxygen uptake during late exercise or early recovery ²⁶.

Echocardiographic examination

Two-dimensional, M-Mode and Doppler echocardiography were performed by physicians (vivid-i, GE Medical Systems, Waukesha, Wisconsin, WI, USA) as described in detail elsewhere ²⁸. Measurements of RVEDD, RVOT, PVAT and TAPSE were performed according to the guidelines of the American Society of Echocardiography ^{29,30}. MPAP was calculated in mmHg using the following equation:
 $MPAP = 10^{(-0.0068 * \text{pulmonary valve acceleration time [PVAT]} + 2.1)}$.

Cardiac MR Imaging and Analysis

Cardiac MR imaging was performed on a 1.5-T MR system (Magnetom Avanto; Siemens Medical Systems, Erlangen, Germany) as previously described ³¹. Quantitative image analysis was performed by two observers with 3 and 5 years of cardiac MR imaging experience using semiautomatic tools in QMass MR 7.2 (MEDIS, Leiden, Netherlands). Interobserver variability was computed in a random subsample of 5%. Certification examinations for interobserver variations revealed an agreement of >90% ²⁸. Postcontrast images were interpreted by the two readers mentioned above and supervised in a consensus reading by a radiologist with 12 years of experience. All observers were unaware of the participants' medical history.

For the RV measurements, RVEDV and RVESV were manually traced in end-diastole and end-systole in transverse axis view. Volumes below the pulmonary valve were included. At the inflow tract, thin-walled structures without trabeculations were not included as part of the RV. RVEDV was determined during the first image of the acquisition. RVESV was measured by determining the phase in which the RV intracavity blood pool was at its smallest by visual assessment at the midventricular level. RVSV, RVCO and RVEF were calculated following the equations below:

$$\text{RVSV (ml)} = \text{RVEDV} - \text{RVESV}$$

$$\text{RVCO (l/min)} = \text{RVSV} \times \text{heart rate} \times 0.001$$

$$\text{RVEF (\%)} = (\text{RVEDV} - \text{RVESV}) / \text{RVEDV}$$

Statistical Analyses

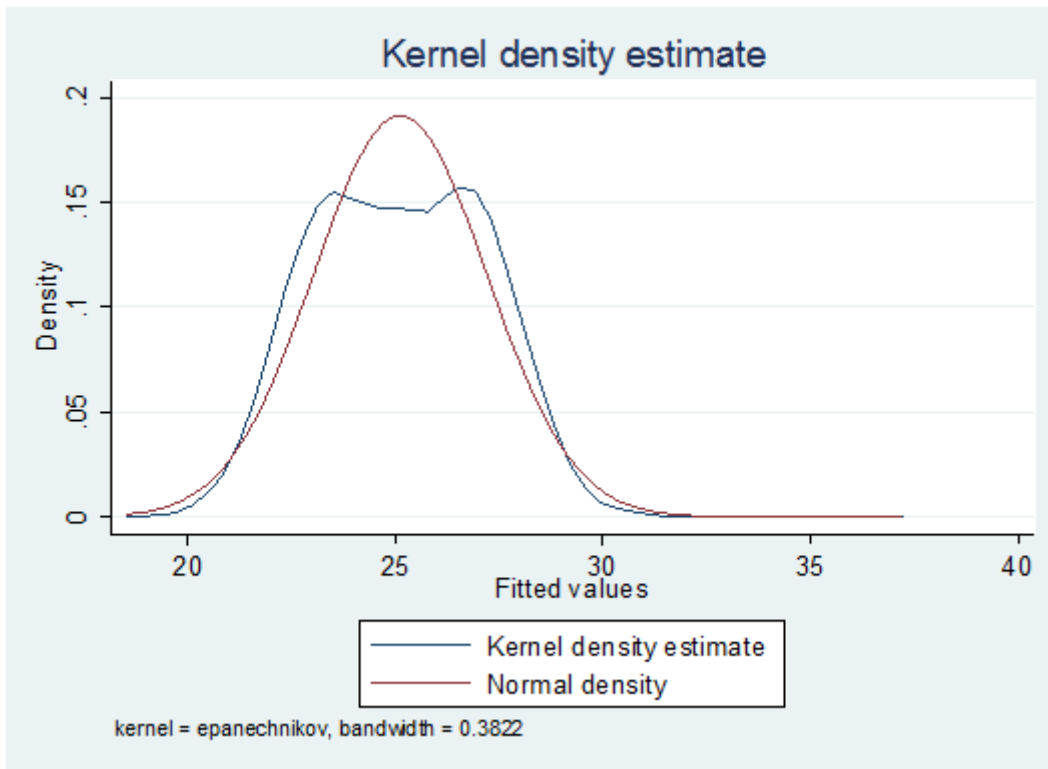
To characterize the study sample, data is reported as the median (25th and 75th percentile) for continuous variables and as percentages for categorical variables stratified by quartiles and sex. The p for trend was calculated by univariate linear regression models with the continuous $\text{VO}_{2\text{peak}}$ variable as outcome and each of the listed variables as explanatory variables. The association of $\text{VO}_{2\text{peak}}$ with RV parameters was investigated by linear regression models adjusted for age, sex (not when stratified by sex), body fat mass, height^{2.7}, systolic BP, use of antihypertensive medication, glycated hemoglobin, use of hypoglycemic medication, smoking status and eGFR. In order to evaluate the robustness of our findings in light of dropout from baseline to follow-up examination (SHIP-0 to SHIP-2) and individuals that did not take part in the echocardiographic and MRI examinations, we performed inverse probability weighting³², assuming a missing at random mechanism³³, based on sociodemographic and health-related variables in our analyses. Inverse-probability weights were applied to consider drop-outs of individuals between SHIP-0 and SHIP-

2 and between the basic and the CPET examinations. The intention behind these weights is to weight up the impact of individuals from groups, who are more likely to drop out of the study, and to weight down the impact of individuals from groups, who are less likely to drop out, in the regression analyses. To calculate these weights we used logistic regression models with participation at the CPET-examination as outcome and sociodemographic, behavioral, and cardiovascular risk factors from the core examinations as explanatory variables. For SHIP-2 participants, we additionally computed weights for the drop-out from SHIP-0 to SHIP-2 and multiplicatively combined these weights with the CPET-weights. With this approach, we aimed to improve the representativeness of our analyses.

We used fractional polynomials to test potentially non-linear relationships between exposure and outcomes³⁴. A two-sided p-value $p < 0.05$ was considered as statistically significant. Statistical analyses were performed using Stata 14.1 (Stata Corporation, College Station, TX, USA).

The choice of the covariates was based on the published literature considering variables available in our dataset that might potentially confound the association between VO_{2peak} and cardiac structure and function. We choose covariates for confounder control that are 1. causes of the outcome, 2. causes of the exposure, but 3. not covariates that are instrumental variables (i.e. instrumental variables affect the outcome only through the exposure and have no direct effect on the outcome)⁵⁷.

The model assumptions were verified and confirmed.



We tested for multicollinearity. There was collinearity between age and eGFR (-0.65), but we decided to keep our original adjustment because of the effects of kidney function on CRF and heart geometry and function as suggested by previous cardiovascular studies. On the other hand, the exclusion of eGFR did not lead to a major change in our results (please see below).

Parameter	With eGFR	Without eGFR
	β -coefficient (95% CI), p-value	β -coefficient (95% CI), p-value
RVEDD (mm)	1.18 (0.66 to 1.71), p<0.001	1.16 (0.63 to 1.69), p<0.001
RVEDV (ml)	23.5 (18.7 to 28.4), p<0.001	23.5 (0.18.7 to 28.4), p<0.001

Table S1. Characteristics of the study sample stratified by whole sample and analyses sample.

Parameter	Whole sample	Analyses sample
N (%)	6,753	2,844
Age (years)	54 (42, 66)	51 (41, 62)
Women (%)	52.0	52.3
VO₂peak (l/min)	1.84 (1.48, 2.36)	1.90 (1.51, 2.42)
Fat-free mass (kg)	54.9 (46.8, 65.9)	54.6 (46.7, 65.7)
Fat mass (kg)	22.5 (17.5, 29.0)	21.4 (17.0, 27.1)
Body mass index (kg/m²)	27.6 (24.6, 31.1)	26.8 (24.2, 29.8)
Systolic blood pressure (mmHg)	129 (116, 142)	127 (115,138)
Diastolic blood pressure (mmHg)	77.5 (70.5, 84.5)	77.0 (71.0, 84.0)
Hypertension (%)	55.0	45.4
Glycated hemoglobin (%)	5.30 (4.90, 5.70)	5.30 (4.90, 5.60)
Diabetes mellitus type 2 (%)	13.4	7.81
Total cholesterol (mmol/l)	5.40 (4.60, 6.20)	5.40 (4.70, 6.20)
LDL-cholesterol (mmol/l)	3.30 (2.66, 3.95)	3.36 (2.75, 3.97)
HDL-cholesterol (mmol/l)	1.39 (1.15, 1.66)	1.43 (1.20, 1.70)
Cholesterol-hdl ratio	3.83 (3.13, 4.73)	3.77 (3.09, 4.63)
Estimated glomerular filtration rate (ml/min/1.73 m²)	88.8 (74.8, 101)	91.7 (79.1, 103)
Smoking (%)		
Never	37.2	38.8
Current	24.8	21.7
Former	38.0	39.5
Physical inactivity (%)	31.3	26.6

Data are medians (25th, 75th percentile) or percentage.

VO_{2peak} - maximal oxygen uptake, LDL-cholesterol – low-density lipoprotein cholesterol, HDL-cholesterol – high density lipoprotein cholesterol.

Table S2. Adjusted* β -coefficient (95% CI) of the associations of peak oxygen uptake (VO_{2peak}) with echocardiographic and cardiac magnetic resonance imaging derived parameters in pooled age analyses and stratified by age.

Parameter	Overall			≤ 50 years			> 50 years		
	β coefficient (95% CI)	p-value	R ²	β coefficient (95% CI)	p-value	R ²	β coefficient (95% CI)	p-value	R ²
Right ventricular structural parameters based on echocardiography									
RVEDD (mm)	1.18 (0.66 to 1.71)	<0.001	0.16	1.09 (0.47 to 1.71)	0.001	0.19	1.17 (0.30 to 2.05)	0.009	0.13
RVOT (mm)	1.41 (0.90 to 1.92)	<0.001	0.25	0.83 (0.28 to 1.39)	0.003	0.30	1.92 (1.04 to 2.80)	<0.001	0.21
Functional right ventricular parameters based on echocardiography									
MPAP (mmHg)	-0.97 (-1.71 to -0.22)	0.011	0.17	-0.83 (-1.59 to -0.07)	0.033	0.16	-1.09 (-2.45 to 0.27)	0.115	0.08
TAPSE (mm)	0.84 (0.46 to 1.22)	<0.001	0.05	1.05 (0.55 to 1.55)	<0.001	0.07	0.71 (0.12 to 1.29)	0.019	0.05
e'/a' ratio	-0.016 (-0.062 to 0.030)	0.499	0.10	0.002 (-0.042 to 0.045)	0.942	0.14	-0.001 (-0.096 to 0.095)	0.991	0.02
Functional and structural right ventricular parameters based on cMRI									
RVEDV (ml)	23.5 (18.7 to 28.4)	<0.001	0.53	20.9 (15.6 to 26.2)	<0.001	0.52	29.6 (21.2 to 38.0)	<0.001	0.52
RVESV (ml)	13.0 (9.81 to 16.2)	<0.001	0.49	11.6 (8.10 to 15.1)	<0.001	0.47	16.4 (11.0 to 21.8)	<0.001	0.49
RVSV (ml/beat)	10.7 (8.10 to 13.3)	<0.001	0.40	9.26 (6.19 to 12.3)	<0.001	0.39	13.2 (8.52 to 17.9)	<0.001	0.37
RVCO (l/min)	0.58 (0.36 to 0.79)	<0.001	0.40	0.44 (0.21 to 0.68)	<0.001	0.35	0.80 (0.39 to 1.20)	<0.001	0.36
RVEF (%)	-0.91 (-1.96 to 0.15)	0.092	0.17	-0.66 (-1.78 to 0.47)	0.253	0.14	-1.78 (-3.62 to 0.06)	0.058	0.21

Adjusted for age, sex, body fat mass, systolic blood pressure, use of antihypertensive medication, glycated hemoglobin, use of hypoglycemic medication, smoking status and estimated glomerular filtration rate (CKD-EPI formula). Data was weighted according to dropout from baseline to follow-up examination (SHIP-0 to SHIP-2) and individuals that did not take part in the echocardiographic and MRI examinations (SHIP-2 and SHIP-Trend). RVEDD – right ventricular end-diastolic diameter, RVOT – right ventricular end-diastolic outflow tract diameter, MPAP – mean pulmonary arterial pressure, TAPSE – tricuspid annular plane systolic excursion, e´/a´ ratio – lateral early and late tricuspid annular peak diastolic velocity ratio, RVEDV – right ventricular end-diastolic volume, RVESV – right ventricular end-systolic volume, RVSV – right ventricular stroke volume, RVCO –right ventricular cardiac output, RVEF – right ventricular ejection fraction