Reference Values for Cardiopulmonary Exercise Testing in Healthy Volunteers-the

SHIP Study

Short running title: Cardiopulmonary Exercise Testing in Adults

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Abstract

Cardiopulmonary exercise testing (CPET) is a widely applied clinical procedure. The study aim was to acquire a comprehensive set of reference values for cardiopulmonary responses to exercise and to evaluate possible associations with gender, age and body mass index.

A standardised progressive incremental exercise protocol on a cycle ergometer was applied to 1708 volunteers of a cross-sectional epidemiologic survey, called "Study of Health in Pomerania – SHIP". Individuals with cardiopulmonary disorders, echocardiographic or lung function pathologies were excluded. The influence of potential confounding factors, such as smoking, taking beta-blockers, hypertension, diastolic dysfunction, BMI, and physical activity, were analysed for their influencing power. Reference values of CPET parameters were determined by regression analyses.

Of the volunteers, 542 current smokers and obese individuals were excluded for not being representative of a healthy population. The final sample size was 534 (253 males), ages 25 to 80 years. The study provides a representative set of reference values for CPET parameters based on age and weight.

Gender and age have a significant influence on exercise parameters. While addressing the problem of a selection bias, this study provides the first comprehensive set of reference values obtained in a large number of healthy volunteers within a population-based survey.

Introduction

Cardiopulmonary exercise testing (CPET) is a widely applied routine procedure in daily clinical work, used for the investigation of cardiac and pulmonary disorders. Due to their prognostic and therapeutic implications [1, 2], the normalcy of response to incremental exercise is usually considered with respect to particular functional indices, such as the peak oxygen uptake ($V'O_2$,peak), the estimated lactate threshold (θL), as well as the characterisation of ventilatory efficiency expressed by the minute ventilation changes as a function of the pulmonary CO₂ output (V'E-V'CO₂ slope). Reference values for these indices have been established, usually with respect to gender, age, body features and physical activity, by several investigators [3-5]. The interpretation of the results of exercise tests requires knowledge of the normal response [6], but a consensus has not been reached on the definition of normalcy. The commonly used reference values are based on exercise capacities of shipyard workers [3] or university members [4]. "Healthy volunteers" were usually defined as subjects without self-reported evidence or clinical symptoms and signs of heart and lung disease, a normal resting electrocardiogram (ECG), a normal 12-lead ECG response to an exercise stress test, and the absence of any medication, especially any with cardiorespiratory effects. However, obtainable reference datasets show relevant limitations, considering their sample recruitment, age distribution, the type of exercise protocol, as well as the lack of consideration of echocardiographic and lung function data.

The aim of this study was to acquire a comprehensive set of reference values on gas exchange and exercise capacity in cardiopulmonary exercise in a representative, healthy population. To minimise selection bias for the study population, a standardised progressive incremental exercise protocol on a cycle ergometer was performed by healthy subjects recruited from a large-scale, cross-sectional, population-based cohort in Northeast Germany.

Subjects and Methods

Study population

Study volunteers consisted of participants of the Study of Health in West Pomerania (SHIP). SHIP is a cross-sectional, population-based survey in a region in the northeast of Germany. The study details are given elsewhere [7]. In brief, from the entire study population of 212,157 inhabitants living in the area, a sample was selected from the population registration offices, where all German inhabitants are registered. A two-stage cluster sampling method was adopted from the WHO MONICA Project Augsburg, Germany [8]. A representative sample was drawn, comprised of 7008 adults, ages 20 to 79 years with 292 persons of each gender in each of the twelve 5-year age strata. The net sample (without migrated or deceased people) consisted of 6267 eligible subjects, of whom 4310 individuals eventually participated in the baseline study of SHIP (SHIP-0). Data collection started in October 1997 and was finished in March of 2001. From March 2003 until July 2006, a 5-year follow-up examination was performed (SHIP-1). The sample (without migrated, deceased or non-responding people) now compromised 3300 subjects (1584 males, 1709 females) ages 25 to 85 years. Of those, 1708 individuals (834 males, 874 females) volunteered for a standardised progressive incremental exercise test on a cycle ergometer.

All participants were investigated in health examination centres established for the purpose of the study and gave written informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the University of Greifswald.

Pre-exercise diagnostics and exclusion criteria

Socio-demographic and behavioural characteristics, as well as information on medical histories, were assessed using computer-assisted personal interviews, which were administered by trained and certified staff. Previous history of diseases was based on self-

reported physician's diagnosis. The definition of cardiopulmonary disorders was based on self-reported physician's diagnosis, use of specific medication (based on the anatomic, therapeutic, and chemical [ATC] code) [9], electro- or echocardiographical pathological findings, and lung function abnormalities measured by spirometry and bodyplethysmography. Normal lung function was defined according to recommendations of the ECSC [10]. All clinical tests were performed by experienced, trained and certified physicians. All examiners were trained in an experienced clinical department before entering the study. Initial certification was awarded to potential observers after a minimum of 3 months of training. Observers were held to strict quality criteria. To facilitate comparability between SHIP and other population-based studies in Germany, external observers were regularly invited to participate in certification procedures. The data collection phase was monitored by a Data Safety and Monitoring Committee.

Height and weight were measured for the calculation of the body mass index (BMI = body weight (kg) / height² (m²). Systolic and diastolic blood pressure were measured three times in seated subjects after a 5 minute rest period, with each reading being followed by an additional rest period of 3 minutes. Mean blood pressure was calculated from the last two measurements. A 12-lead electrocardiogram was taken of each participant. The medication was recorded by a computer-aided method using the ATC code. A blood sample was drawn from the cubital vein in the supine position.

Subjects with the following criteria were excluded from the present study: past myocardial infarction, signs of ischaemia/infarction, right or left bundle branch block or Wolff-Parkinson-White syndrome in the electrocardiogram, the presence of a cardiac pacemaker, stenosis or insufficiency of the cardiac valves, systolic and diastolic failures as well as a cardiac shunt vitium found in the echocardiography, pulmonary diseases, bronchial asthma, chronic obstructive bronchitis (ratio of forced expiratory volume over one second (FEV1) to forced vital capacity (FVC) being < 0.7 and the FEV1 being < 80%pp [11]), or emphysema

(intra- thoracic gas volume [ITGV] being > 140%pp, residual volume [RV] % of total lung capacity [TLC] being > 140%pp), all defined by spirometric/bodyplethysmographic criteria and/or patients self report, neuromuscular or musculoskeletal disorders based on neurological examination, anaemia and/or – with the exception of beta blockers - the use of drugs with influence on cardiopulmonary function. Patients with a breathing reserve (minute ventilation/maximum voluntary ventilation [VE/MVV %]) > 0.8 (see below) were excluded from the study.

Exercise testing

A symptom-limited exercise test using one calibrated electromagnetically braked cycle ergometer with an electrical seat height adjustment (Ergoselect 100, Ergoline, Germany) was performed according to a modified Jones protocol (stepwise increase in work load of 16 Watts/minute, starting with unloaded cycling plus the ergometer related permanent load) [4]. Gas exchange and ventilatory variables were analysed breath by breath using a computer based system. Each protocol was preceded by a resting period of at least 3 minutes to reach steady state conditions [steady state status was analysed for oxygen uptake ($V'O_2$ ml/min), carbon dioxide output ($V'CO_2$ ml/min), minute ventilation (V'E I/min), ventilatory equivalents for oxygen and carbon dioxide ($V'E/V'O_2$ and $V'E/V'CO_2$), end tidal gas concentrations for oxygen and carbon dioxide ($VEIO_2$ and $VEIO_2$).

The procedure was continuously monitored by a physician. In the absence of chest pain and ECG abnormalities, all tests were continued as symptom-limited (volitional exertion, dyspnoea or fatigue). Prior to the test, patients were encouraged to reach maximal exhaustion, while during exercise no further motivational interventions were utilised.

All tests were performed at room air according to current guidelines for exercise testing, with continuous monitoring of ECG, blood pressure and oxygen saturation [12, 13].

Gas exchange variables

Respiratory gas exchange variables were measured continuously throughout the resting period, the unloaded cycling period, and the exercise test using a VIASYS HEALTHCARE system (Oxycon Pro, Rudolph's mask). Prior to each test, the equipment was calibrated in standard fashion with reference gas and volume calibration. A standard 12-lead ECG was obtained at rest, at every minute during exercise, and for at least 5 minutes during recovery; blood pressure was measured with a standard cuff sphygmomanometer. V'E (l/min), tidal volume (VT [1]), V'O2 and V'CO2 (ml/min) were acquired on a breath-by-breath basis and averaged over 10-second intervals. V'O2,peak was defined as the highest 10-second average of V'O2 in the last minute of exercise. The ventilatory equivalent for CO2, expressed as the relation of V'E to V'CO2, was assessed at rest, at lactate threshold and peak exercise. In addition, the slope of the regression of both parameters (V'E-V'CO2 slope) excluding excess hyperventilation at the end of exercise was assessed [2]. The lactate threshold was determined according to Wasserman et al. [6]. The breathing reserve (V'E/MVV) was calculated as peak V'E in relation to MVV. MVV was calculated by the forced expiratory volume over one second (FEV1) x 41 [14].

Statistical analysis

The data were separately analysed for each age and gender stratum. The age strata were graded into five groups: 1 = 25-34, 2 = 35-44, 3 = 45-54, 4 = 55-64 and $5 \ge 64$ years of age. Logistic regression analyses were done to compare the participants of cycle ergometry with the entire SHIP-1-cohort in order to detect a selection bias. Analysis of variance adjusted to gender and age was performed in order to evaluate the influence of specific confounding factors on selected parameters such as $V'O_2$, peak and oxygen uptake at the lactate threshold ($V'O_2$ at θ L), both adjusted for weight. Analysis of variance was performed on both the

absolute values of $V'O_2$, peak and $V'O_2$ at θL (ml/min) and the body weight-corrected results (ml/min/kg).

For the evaluation of reference values, quantile regression analysis, a statistical method for estimating models for the conditional quantile functions [15], adjusted for age, gender and body mass index was performed. Since quantile regression makes no distributional assumption, an initial transformation of the original data was unnecessary.

Statistical significance was defined as p<0.01. Values are given as median, odds ratio (OR) and 5%, 95% confidence intervals (CI). The participants were divided into a normal weight and an overweight group (BMI \leq 25 kg/m² and BMI > 25 kg/m² but < 30 kg/m²).

All statistical analyses were performed with SPSS software, version 15.0 (SPSS GmbH Software, Munich, Germany) or SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, USA). The graphics were done with SigmaPlot, version 9.0 (Systat Software, Inc., USA).

Confounding factors

The following variables were statistically examined for a possible impact on exercise parameters:

- 1. Smoking habits, classified as a) non-smoker; b) current smoker; c) former smoker.
- 2. Beta-blocker medication.
- 3. Hypertension at rest, defined according to the WHO standards as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg at rest or self reported use of antihypertensive medications [16].
- Diastolic dysfunction (E/A ratio < 1 (≤50 years) or < 0.5 (>50 years) and no systolic dysfunction [17]).
- 5. BMI (BMI < 19 kg/m^2 (underweight); BMI 19-25 kg/m² (normal weight); BMI 25-30 kg/m² (overweight); BMI > 30 kg/m^2 (obesity) (definition based on current WHO-classification).

6. Exercise habits, classified as a) less than 1 hour weekly; b) 2-3 hours weekly; c) 3-4 hours weekly and d) more than 4 hours weekly.

Results

Selection of the reference population

Relevant characteristics and distributions of all study participants are demonstrated in Table 1. The mean age of the participants was 52.02 (SD \pm 13.57) years.

Table 1. - Descriptive statistics of the complete study sample who underwent cycle ergometry prior to the exclusion of confounding factors.

Age categories	Sex	N	Total N and (%)	BMI mean ± SD	Smokers (%)	Hypertension (%)	Beta- blocker (%)
25-34	f	112		27.7 ± 5.2	51.8	4.5	6.3
	m	95	207 (12.2)	26.5 ± 3.8	63.1	22.1	5.3
35-44	f	178		25.5 ± 4.4	57.8	13.5	4.5
	m	179	357 (20.9)	27.1 ± 3.7	62.6	34.6	6.1
45-54	f	207		27.8 ± 5.2	50.7	27.1	20.3
	m	176	383 (22.4)	28.8 ± 4.5	75.0	42.0	14.8
55-64	f	218		28.6 ± 4.9	33.9	32.6	29.4
	m	185	403 (23.6)	28.5 ± 3.7	70.8	47.0	30.3
65-74	f	136		28.7 ± 4.7	23.5	39.7	39.7
	m	148	284 (16.6)	29.3 ± 4.2	76.3	50.7	41.2
> 75	f	23		29.0 ± 3.9	17.4	35.0	56.5
	m	51	74 (4.3)	27.7 ± 3.5	74.5	50.9	35.3
Total	f	874		27.3 ± 5.1	43.0	24.9	21.5
	m	834		28.1 ± 4.1	70.3	41.4	21.2

Mean data, standard deviations and percentages are presented. Smokers are defined as current smokers. Hypertension is defined according to the WHO standards as systolic blood pressure \geq 140 mmHg or diastolic blood pressure \geq 90 mmHg at rest. SD: standard deviation;

N: absolute number of participants; BMI: body mass index (kg/m²); f: female; m: male.

Confounding factors on the CPET parameters

The following factors were examined for their influence on $V'O_2$, peak and $V'O_2$ at θL (as absolute values and corrected for body weight) both adjusted for gender and age by analysis of variance.

According to their smoking habits, a significant difference was proved between the current and the former smokers as well as the non-smokers. There was no difference found between the non- and former smokers. Consequently, current smokers were excluded from the study. The beta-blocker use did not prove to be significant for all variables. Therefore, all participants with beta-blocker medication were included in the study. Arterial hypertension did not prove to be of significant influence on the selected CPET parameters. Hypertensive participants were included. No significant influence on the selected parameters was found concerning diastolic dysfunction. Therefore, this was not an exclusion criterion. Obese participants (BMI $> 30~{\rm kg/m^2}$) had to be excluded from the study, since we found a significant influence on the CPET parameters comparing normal to obese volunteers for all variables. The influence of physical activity was not consistently significant throughout the investigated groups. Therefore, physical activity was not an exclusion criterion.

In conclusion, current smokers (n = 265), obese participants (n = 225), and obese smokers (n = 52) were excluded from the study sample. In total, 632 individuals were excluded for medical reasons. The final sample size was 534 (253 male, 281 female) of the initial 1708 participants in CPET. The age distribution of the study sample is shown in Figure 1.

Comparison between the complete SHIP-1 population and CPET participants

We found a significant difference (p = 0.025) between the participants and the non-participants in CPET with more non-smoking volunteers participating in CPET. We also found significantly fewer hypertensive people among the participants in CPET (p < 0.01). No

significant difference was found between the participants in CPET and the non-participants with regard to BMI status and the occurrence of pulmonary diseases (p = 0.14).

Exercise protocol

The main reasons for termination of exercise in the present study were muscular exertion and fatigue (72.9 %) as well as dyspnoea (27.1 %). The mean exercise duration of our study sample, subdivided into 10-year age categories, is shown in Table 2; it was approximately 11 minutes in the 25-34 year age group and 7.8 minutes for the individuals older than 65.

Table 2. - Mean exercise durations subdivided into 10-year-age categories.

10-year-age categories of	Mean		Standard deviation
participants	(minutes)	N	(minutes)
25-34	11.02	92	3.36
35-44	11.29	145	3.43
45-54	9.31	115	2.86
55-64	9.08	120	2.73
65-74	7.81	55	2.23
>=75	7.98	7	1.16
Total	9.92	534	3.24

Mean data and standard deviations are presented. N: absolute number of participants.

Reference values

 $V'O_2$, peak and $V'O_2$ at θL

In the investigated study sample, $V'O_2$, peak revealed a significant dependency on age, gender and BMI. $V'O_2$, peak declined with advancing age and increasing BMI, and was lower for women in comparison to men (Figure 2). The effect of age, gender and BMI can be calculated by a predictive equation shown in Table 3.

In the male population with a BMI of \leq 25 kg/m², oxygen uptake at the lactate threshold ($V'O_2$ at θL) averaged 54% (5th percentile) and 60% (95th percentile) respectively of $V'O_2$,peak. In those with a BMI of > 25 kg/m² and < 30 kg/m² the $V'O_2$ at θL averaged 52% (5th percentile) and 64% (95th percentile) of the $V'O_2$,peak. In the female population with a BMI of \leq 25 kg/m² the $V'O_2$ at θL was 55% (5th percentile) and 62% (95th percentile) of the $V'O_2$,peak. Females

with a BMI of $> 25 \text{kg/m}^2$ and $< 30 \text{kg/m}^2$ had an average $V'O_2$ at θL of 53% (5th percentile) and 62% (95th percentile) of the $V'O_2$, peak.

Although there was a wide range of $V'O_2$ at $\theta L / V'O_2$, peak %, within the age groups, no value less than 41% was found. Within both genders, there was no significant dependency of $V'O_2$ at θL on age or BMI, particularly according to the 5th percentile, whereas there seemed to be an age-correlated decline within the 95th percentile. This correlation is shown in Figure 3. The effect of age, gender and BMI can be calculated by a predictive equation shown in Table 3.

Table 3. - Equations for reference values for peak oxygen uptake, oxygen uptake at lactate threshold and oxygen pulse, all corrected for body weight, based on the study sample (n=534).

V´O ₂ ,peak (ml/min/kg)	intercept	age	age ²	sex	BMI	age*BMI	sex*BMI	age*sex	age*sex*BMI
5% median 95% V´o₂ at θ∟ (ml/min/kg)	30.9643 47.7565 61.3721 intercept	-2.5661 -0.9880 -1.9479 age	-0.0263 -0.2356 -0.3053 age ²	-3.7224 -8.8697 -9.1229 sex	1.8765 2.3597 3.8892 BMI	0.1082 -2.0308 -1.9492 age*BMI	-2.9703 -3.7405 -6.7455 sex*BMI	0.7361 0.2512 0.0716 age*sex	0.2799 1.3797 1.6900 age*sex*BMI
5% median 95% V´O ₂ /HR (ml/beat)	8.0266 18.3219 31.4007 intercept	2.1582 0.3399 -0.4891 age	-0.2337 -0.0369 0.0681 age ²	0.9685 -1.1117 -2.7167 sex	-0.9729 1.5436 12.5034 BMI	0.4670 -0.9756 -3.4748 age*BMI	-0.5278 -1.8656 -7.0634 sex*BMI	-0.5030 -0.2643 -0.7041 age*sex	-0.1150 0.5895 1.5570 age*sex*BMI
5% median 95% Entries are	14.8333 22.1667 29.2583	0.5583 -0.8167 -0.0333	-0.2417 0.0167 -0.1250	-4.6667 -5.8667 -7.6917	2.4083 4.8897 1.6980	-0.3583 -1.4230 -1.0729	-0.1333 -2.4230 -0.3397	0.5167 0.3333 0.0917	-0.0167 0.7897 0.7146

entering subjects' characteristics, sex is coded as 1 for males and 2 for females. BMI is coded as 0 for a BMI \leq 25 kg/m² and 1 for a BMI > 25 kg/m². The age was sorted into 5 age groups: 1 = 25-34, 2 = 35-44, 3 = 45-54, 4 = 55-64 and $5 \geq 64$. The content of each column was multiplied with the coded characteristics. To receive the 5%, median and 95% reference ranges the contents of each column have to be added. BMI: body mass index (kg/m²); $V'O_2$, peak: peak oxygen uptake per kilogram body weight (ml/min/kg); $V'O_2$ at θ L: oxygen uptake at the lactate threshold per kilogram body weight (ml/min/kg); $V'O_2$ /HR: oxygen pulse (ml/beat).

V'E/V'CO₂ at rest and lactate threshold and V'E-V'CO₂ slope

percentile in men, versus 26.7 (SD 0.64) for the 5th percentile and 39 (SD 1.6) in women. With advanced age, an increase among the reference values was found. No value above 50 was observed in either men or women. The corresponding equation can be found in Table 4. The *V'*E/*V'*CO₂ at lactate threshold (*V'*E/*V'*CO₂ at θL) was 22.3 (SD 0.6) for the 5th percentile and 30.6 (SD 0.8) for the 95th percentile in men, versus 22.3 (SD 0.52) for the 5th percentile and 31.8 (SD 1.2) in women. With advancing age, an increase among the reference values was found. No value above 35 was observed in either men or women. The corresponding equation can be found in Table 4.

The V'E/V'CO₂ at rest was 27.7 (SD 0.7) for the 5th percentile and 42.3 (SD 1.2) for the 95th

Ventilatory efficiency during exercise was not different with respect to gender. In contrast, an age-correlated increase within the reference values was found. For men, the $V'E-V'CO_2$ slope increased from 18.00 (SD 0.9) for the 2.5th percentile and 30.00 (SD 1.50) for the 97.5th percentile at mean age 29 (25-34) years to 21.00 (SD 1.30) for the 2.5th percentile and 34.00 (SD 3.15) for the 97.5th percentile at mean age > 64 years (Figure 4).

Aside for the differences according to the standard deviations, similar values were found for women. The corresponding equation can be found in Table 4.

Further ventilatory results

Calculations for additional reference values, such as breathing frequency (BF), V'E (l/min), VT (l), V'E/MVV, oxygen pulse ($V'O_2/HR$ ml/beat), and end tidal gas concentration for carbon dioxide (PETCO₂ mmHg) are shown in Table 4, for the oxygen pulse depicted in Table 3.

Table 4. - Equations for the calculation of the investigated parameters of CPET, based on the study sample (n=534).

	intercept	age	age ²	sex	age*sex
BF (I/min)	-				-
2.5%	6.2563	-0.7587	0.2523	2.0143	-0.1423
median	18.9402	-2.3410	0.2148	-0.6465	0.6565
97.5%	28.1137	4.6633	-0.9750	-0.8463	-0.1097
<i>V</i> T (I)					
2.5%	0.4923	0.0180	0.0038	-0.0240	-0.0260
median	1.1218	0.0309	0.0018	-0.1816	-0.0201
97.5%	1.2752	0.7346	-0.1087	-0.0310	-0.0930
V´E (l/min)					
2.5%	71.1250	-4.000	-0.1250	-15.2500	1.2500
median	128.5	-5.0	-0.5	-35.5	2.5
97.5%	151.862	12.5230	-2.4770	-28.8163	-4.0919
V'E/MVV (%)					
2.5%	15.2250	-1.300	0.6250	17.7750	-2.7500
median	126.5631	-9.3185	-0.4303	-22.4095	2.8023
97.5%	171.200	4.300	-1.500	-28.600	-1.200
V'O ₂ ,peak (ml/min)					
5%	2966.00	-192.00	-3.3333	-800.00	76.6667
median	4307	-241	-20	-1281	133
95%	6107.67	-502.67	-8.3333	-1124.33	177.6667
V'O ₂ at θL (ml/min)					
5%	1033.33	66.6667	-16.6667	-233.333	16.6667
median	1841.723	-45.943	-3.6219	-411.439	15.3597
95%	4143.33	-405.00	-8.3333	-1310.00	180.000
V'E/V'CO ₂ at rest					
5%	24.000	2.1667	-0.1667	0.5000	-0.5000
median	29.9167	1.9583	0.1250	1.4583	-1.4583
95%	33.1667	4.1667	0.0000	2.1667	-1.8333
V'E/V'CO ₂ at θL					
5%	16.6667	2.5000	-0.1667	1.5000	-0.5000
median	20	2	0	3	-1
95%	25.4167	1.6667	-0.0833	0.9167	0.0833
V´E-V´CO ₂ slope	10.40=0	4 500	0.0==0	0.000	0.000
2.5%	19.1250	-1.500	0.3750	0.000	0.000
median	22.0	-1.5	0.5	2.5	-0.5
97.5%	30.6667	-1.000	0.3333	0.000	0.000
PETCO ₂ at rest					
(mmHg)	26.00	2 405	0.4075	2 0075	0.0075
2.5%	36.00	-3.185	0.1875	-3.9375	0.9375
median	38.0312	-1.0000	-0.0938	-3.7812 1.7092	1.0625
97.5%	40.5833	-0.0417	-0.0417	-1.7083	0.2083
PETCO ₂ at θL					
(mmHg)	24 6075	0.6075	0.0027	1 2100	0.2405
2.5% median	34.6875	0.6875	-0.0937	1.2188	-0.3125
97.5%	48.75 54.000	-0.75 1.6667	-0.25 0.16667	-6.00 1.1667	1.25
	for males a	-1.6667		-1.1667	-0.3333

Sex is coded as 1 for males and as 2 for females. Age is graded into 5 age groups: 1 = 25-34, 2 = 35-44, 3 = 45-54, 4 = 55-64 and 5 = > 64 years of age. The content of each column has to be multiplied with the coded characteristics. To calculate the 5%, median and 95%, the 2.5% and 97.5% reference ranges respectively, the contents of each column have to be added. BF: breathing frequency (l/min); VT: tidal volume (l); VE: minute ventilation (l/min); VE/MVV:

breathing reserve (%); $V'O_2$,peak: peak oxygen uptake (ml/min); $V'O_2$ at θ L: oxygen uptake at lactate threshold (ml/min); $V'E/V'CO_2$ at rest: ventilatory equivalent at rest; $V'E/V'CO_2$ at θ L: ventilatory equivalent at lactate threshold; $V'E-V'CO_2$ slope: ventilatory efficieny expressed by the minute ventilation changes as a function of the pulmonary CO_2 output; $PETCO_2$ at rest: end tidal gas concentration for carbon dioxide at rest; $PETCO_2$ at θ L: end tidal gas concentration for carbon dioxide at lactate threshold.

Discussion

The present study provides the first set of reference values for CPET compiled by a representative sample of healthy volunteers using the platform of a population based study – the SHIP population. By excluding subjects with known cardiopulmonary disorders or certain cardiovascular risk factors, a disease-free population was selected.

Most previous reports of reference values for cardiopulmonary exercise variables in healthy individuals have been based on small sample sizes and differed significantly with respect to the populations studied and the methods used [3-6, 18-20]. For example, the reference values established by Wasserman et al. [6] are based on data obtained from 77 male shipyard workers with a mean age of 54 years, including some who were smokers, obese, or hypertensive. They were judged to have normal cardiorespiratory systems based on medical history, physical examination, electrocardiogram during rest and exercise, chest-X-ray, pulmonary function test and exercise performance. However, objective parameters, such as echocardiographic or bodyplethysmographic findings, were not taken into account. Jones et al. [4] recruited their participants from the local university and the general population by advertising. These individuals were stratified into five age groups for both genders until a total sample size of 50 for each gender was reached. "Health" was defined by gaining information on medical history, leisure and occupational habits, exercise-related symptoms and pulmonary function tests. Athletes, subjects with serious illness in the past or any chronic

disorders, as well as individuals suffering from hypertension, were excluded. Smokers remained within the study sample. Inbar et al. [19] established reference values for cardiopulmonary responses in 1424 men, ages 20 to 70 years, who underwent an incremental treadmill exercise. Individuals with abnormal ECG-tracings, medical history, or physical or laboratory findings of cardiac, respiratory, metabolic or neuromuscular diseases were excluded from the study. Current or former smokers were included. Again, no echocardiographic and bodyplethysmographic investigation was performed. Reference values were only provided for male volunteers; recruitment methods were not stated.

Statistical results based on the studies mentioned above are, however, less likely to provide a good estimate of population parameters. Therefore, the contribution made by our study lies in the selection of a quantifiably healthy population, involving several types of clinical examination methods, including echocardiography, pulmonary function tests, bodyplethysmography, as well as information gained from the medical history. These rigorous inclusion criteria are responsible for the fact that only 534 of the initial 1708 individuals were includable. Furthermore, the investigation of confounding factors was of importance for the precision of reference values.

Concerning the exercise mode, it should be mentioned that the $V'O_2$, peak measured during treadmill exercise is about 10% higher than that obtained via cycle ergometry [18]. Therefore, the reference values presented here should only be applied to cycling tests.

The advantage of a population-based study lies in the general applicability of the collected data. Nevertheless, reference values are statistical markers of the value of distribution of a specific test. Thus, factors influencing the distribution of these values may also affect reference values and should be taken into account.

Oxygen uptake

The main results suggest that it is important to include gender, age and BMI when predicting $V'O_2$, peak. Previous population-based studies of $V'O_2$, peak in Europe [18, 20] and North America [21, 22] reported remarkably uniform data, despite different study samples and testing protocols.

Using the equation established in the present study, the values for a 35 to 44-year-old male with a BMI of \leq 25 kg/m² are 23.47 ml/min/kg (5% percentile) and 47.27 ml/min/kg (95% percentile). For a 35 to 44-year-old female with a BMI of \leq 25 kg/m², the values are 21.22 ml/min/kg (5% percentile) and 38.29 ml/min/kg (95% percentile). The $V'O_2$,peak predicted by Wasserman et al. [6] for a 40-year-old man with a BMI of 24.22 kg/m² is 36.76 ml/min/kg and for a woman of the same age and BMI is 25.47 ml/min/kg. The $V'O_2$,peak predicted by Jones et al. [4] for a 40-year-old man is 37.4 ml/min/kg and for a woman of the same age it is 28.6 ml/min/kg. The $V'O_2$,peak predicted by Neder et al. [23] for a 40-year-old man, height 170 cm, weight 70 kg is 32.4 ml/min/kg and for a women of the same age, height and weight is 24.91 ml/min/kg.

As presented in Figures 5 and 6, it is obvious that the values for $V'O_{2,peak}$ found in the literature lie within the 5%-95% percentile range of our study. For female subjects especially, a linear approximation towards the lower CI is obvious. This might be due to the selection criteria applied, or the age distribution in the cited studies. By comparing our median values established from our study with those found in literature, a tendency towards higher values in individuals older than 45 years becomes apparent in both genders. This, we assume, is due primarily to age distribution within the investigated studies and secondarily to cohort effects due to changes in individual, behavioural and environmental factors over decades.

The relevance and importance of the lactate threshold for the characterisation of cardiovascular and metabolic abnormalities was established by Wasserman et al. [24] and, later, Weber et al. [25]. In the present study, gas exchange criteria were used in order to

identify the θ L. Our data show a steady decline of the θ L with advancing age. When calculated as the ratio of $V'O_2$ at θ L/ $V'O_2$,peak values between 52% and 64% were found. The lowest values were found in the youngest age group, whereas the highest values emerged in the oldest age group. Thus, it can be postulated that the lactate threshold increases as a percentage of $V'O_2$,peak with advancing age [3, 5]. Jones et al. [4] and Davis et al. [26] have studied the effect of age on the θ L, as well. Although different θ L detection methods were applied, both groups found the absolute θ L declining with age in both genders, but less than the decrease in $V'O_2$,peak. The ratio of $V'O_2$ at θ L/ $V'O_2$,peak tends to increase with age and tends to be higher in women than in men. These findings are in agreement with the data presented here.

Ventilatory equivalent

Ventilation during exercise is primarily associated with metabolic needs and is therefore closely related to $V'\text{CO}_2$ [19]. In order to assess the efficiency of minute ventilation with respect to CO_2 -elimination during exercise, the ventilatory equivalent for CO_2 ($V'\text{E/V'CO}_2$) is used. This study demonstrates that the ventilatory response to CO_2 production during exercise increases with advancing age. The $V'\text{E-V'CO}_2$ slope was significantly correlated to age for both genders. The mean values from previous studies were 31 ± 4.5 for $V'\text{E/V'CO}_2$ [19, 27]: these values are comparable to those found in our sample.

Sullivan et al. [28] found the $V'E/V'CO_2$ at rest to be around 36 and to decrease with exercise to 30. Hansen et al. [3] reported that among 77 men, mean age 54 years, they found a $V'E/V'CO_2$ of 39 \pm 10 at rest and of 29 \pm 4 at lactate threshold. These values are similar to those found in our sample and lie within the 5% and 95% percentile range of our data.

With respect to the CPET protocol applied in this study, it should be emphasised that variations in incremental step size during CPET can significantly affect exercise time and

maximal power. In daily practice, protocols according to the recommendations of Wasserman et al. [6] are widely used to reach exercise durations between 6 and 12 minutes. Recent recommendations for exercise protocol in healthy volunteers are based on relatively small numbers of subjects [29-31], but emphasise that the duration of exercise should be 8 to 17 minutes, as appropriate. Other parameters of importance for clinical judgement and prognosis such as oxygen uptake, exercise ventilation and ventilatory efficiency appear to be less dependent on variations in exercise protocol. Therefore, an individualisation of incremental step size to reach maximal results for the parameters mentioned above does not appear to be mandatory [32].

In order to assess the generaliseability of the presented results, population-specific factors including the relatively high mean BMI (27.4 kg/m² in men and 26.8 kg/m² in women) and the prevalence of cardiopulmonary disorders have to be taken into account [33]. A high prevalence of hypertension is found in West Pomerania [34]. The study may be criticised for not being multi-centric, and therefore, may be considered to be limited in its applicability outside Germany. However, as the British Framingham study has shown, obesity and cardiovascular diseases are growing challenges in the entire western world. The incidence of obesity and nicotine abuse in our study is comparable to those reported in cohorts of other investigations [35]. Therefore, we assume that the reference values presented in this study are applicable to similar study samples found in the western world.

In addition, this study may be criticised for having a potential selection bias towards including younger, healthier and non-smoking subjects, because participation in cycle ergometry was voluntary. However, since the collected values were adjusted for age, gender and BMI and tested against possible confounding factors, the differences between the general German population, the entire SHIP-population, and the participants in the CPET group were negligible.

Since obesity seems to be a major concern and growing challenge worldwide, further investigation on normal values of CPET for obese individuals is surely necessary.

Conclusion

In summary, this study details the cardiopulmonary responses to incremental cycle ergometry exercise tests in normal, healthy subjects. The relatively wide ranges of response patterns indicate that individual responses vary widely. This finding has to be taken into account when analysing CPET data.

Our results demonstrate that gender, age, and anthropometric characteristics should be considered in the assessment of normalcy of dynamic exercise responses.

We have presented the results of this study in several ways, partly to allow the investigator to compare studies in other laboratories with those of the present study and to encourage a flexible approach to the interpretation of exercise test results in general.

The major strength of our study lies in the use of data from a large population-based sample of adults and the guideline of excluding subjects with any known relevant diseases. Another relevant contribution made by the study lies in the weighting of different confounding factors on exercise performance and in the effort that was made to include several medical methods to establish a healthy study sample.

Funding

This work is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by grants from the German Federal Ministry of Education and Research (BMBF, Grants 01ZZ0403, 01ZZ0103), the Ministry for Education, Research and Cultural Affairs as well as the Ministry of Social Affairs of the Federal State of Mecklenburg-West Pomerania. The CMR encompasses several research projects which, share population-based data from the Study of Health in Pomerania (SHIP; http://ship.community-medicine.de).

Acknowledgements

This work is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research, the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. The CMR encompasses several research projects which share population-based data from the Study of Health in Pomerania (SHIP; http://ship.community-medicine.de).

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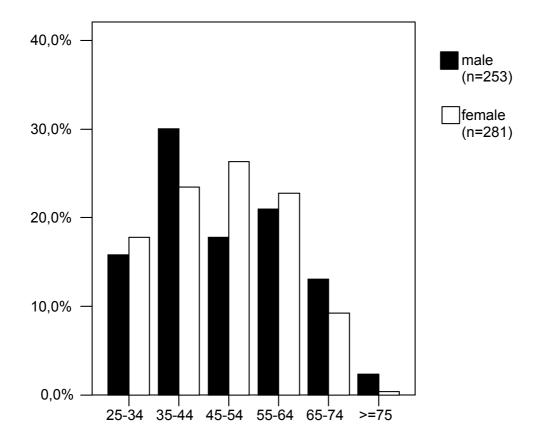


Figure 1: Distribution of the study sample subdivided into 10-year age categories.

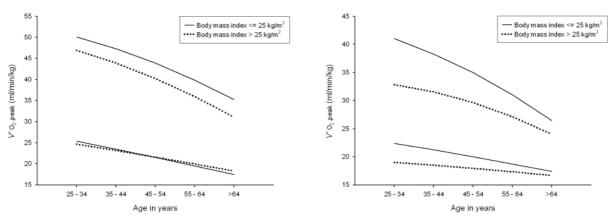


Figure 2: Normal ranges for $V'O_2$, peak (ml/min/kg) for the male (left graph) and female (right graph) population and with relation to age subdivided for BMI groups. Dotted and solid lines present the 5^{th} and 95^{th} percentile.

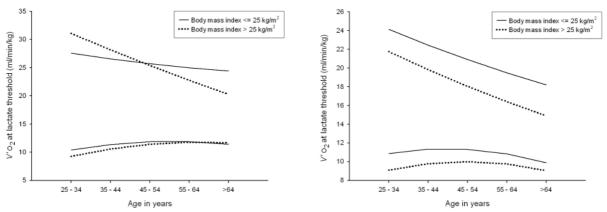


Figure 3: Normal ranges for $V'O_2$ at θL (ml/min/kg) for the male (left graph) and female (right graph) population and with relation to age subdivided for BMI groups. Dotted and solid lines present the 5^{th} and 95^{th} percentile.

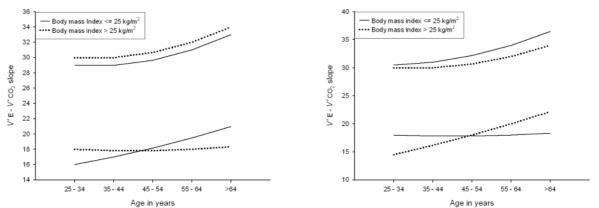


Figure 4: Normal ranges for V'E-V'CO₂ slope for the male (left graph) and female (right graph) population and with relation to age subdivided for BMI groups. Dotted and solid lines present the 5th and 95th percentile.

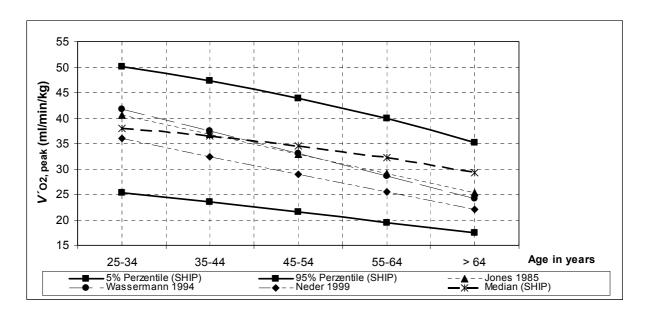


Figure 5: V'O₂,peak (ml/min/kg) subdivided into 10-year-age categories for males. A comparison to historical data was performed for an exemplary individual (height 170 cm, weight 70 kg).

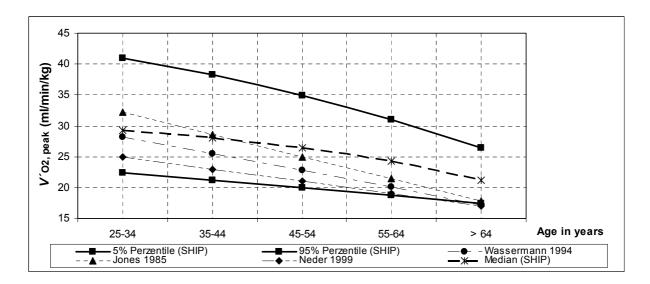


Figure 6: V'O₂,peak (ml/min/kg) subdivided into 10-year-age categories for females. A comparison to historical data was performed for an exemplary individual (height 170 cm, weight 70 kg).