Cardiopulmonary Exercise Testing in Health and Heart Failure – Improving Established Methods and Exploring New Frontiers to Evaluate Physical Fitness Status for Risk Stratification

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Jonathan Wagner
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On application of

Faculty representative	Prof. Arno Schmidt-Trucksäss, M.D.
Primary advisor	Prof. Arno Schmidt-Trucksäss, M.D.
Secondary advisor	Prof. Henner Hanssen, M.D.
External expert	Prof. Leonard Kaminsky, Ph.D.

Basel, October 26, 2020

Prof. Primo Schär, Ph.D.

Dean

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List of Abbreviations

AHA	American Heart Association
$APMHR_{210}$	age-predicted maximum heart rate 210 − age
$APMHR_{208}$	age-predicted maximum heart rate 208 − 0.7 x age
BL	blood lactate
COmPLETE Project	CardiOPuLmonary Exercise TEsting Project
CPET	cardiopulmonary exercise testing
CRF	cardiorespiratory fitness
CV	cardiovascular
CVD	cardiovascular disease
EOV	exercise oscillatory ventilation
FRIEND	Fitness Registry and the Importance of Exercise National Database
HD	health distance
HF	heart failure
HFmEF	heart failure with mid-range ejection fraction
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
LPA	light physical activity
MET	metabolic equivalent of task
MPA	moderate physical activity
NIH	National Institutes of Health
NYHA	New York Heart Association
OUES	oxygen uptake efficiency slope
PA	physical activity
$P_{ET}CO_2$	partial pressure of end-tidal CO_2
RER	respiratory exchange ratio
RFD	rate of force development

VCO_2	carbon dioxide output
VE	ventilation
VO_2	oxygen uptake
VPA	vigorous physical activity
VT	ventilatory treshold

Summary

Background

Aging and the changing age demographics potentially represent one of the most critical problems of our time. The shift of the major causes of morbidity towards chronic disease, coupled with changing age demographics, is likely to lead to an epidemic of age-driven chronic disease. Cardiovascular diseases (CVD), which include heart failure (HF), are the leading cause of death worldwide. The prevalence of HF continues to rise over time in sync with the aging population. HF has been defined as a global pandemic, based on the large number of people affected by the syndrome worldwide. Physiological functions decline with age, and these declines often end in a systemic process that contributes to numerous physiological impairments and disease. The ability to perform physical tasks is critical for maintaining overall functional capacity, and physical fitness parameters are biomarkers of health among older adults, predicting quality of life, disability, and mortality. Links between CVD risk factors and limitations in physical fitness have been widely described. Physical fitness markers include measurements of endurance capacity, muscle strength, and neuromuscular coordination. Cardiorespiratory fitness (CRF) and other markers measured by cardiopulmonary exercise testing (CPET) have been described to be highly useful health markers, and VO_{2peak} is even suggested for use as a vital sign in clinical evaluation. Some limitations, however, likely hinder a wider application of CPET including: 1) the evaluation of whether the true individual physiological limit is reached and VO_{2peak} can be determined, 2) the lack of accurate and suitable normative reference values of healthy individuals across the lifespan, 3) submaximal CPET parameters as an alternative in the case VO_{2peak} was not reached. Further, additional physical fitness measurements and their combination to a composite outcome could provide a useful tool for the management of age-related chronic disease such as HF. To date, however, no attempts to combine biomarkers of several physical fitness domains have been performed.

Aims

The aims of this Ph.D. project were: (1) To review the current evidence of CPET variables for the purpose of risk stratification and management of heart failure with reduced ejection fraction (HFrEF); (2) To design and conduct a study involving a comprehensive assessment of physical fitness components and cardiovascular (CV) function to identify which markers are most highly associated with CV risk with increasing age and which markers are most impaired in HF; (3) To determine data-based and age-dependent cutoffs for maximal exercise criteria for a general population which is free of exercise-limiting chronic conditions; (4) To provide reference values for maximal and submaximal CPET parameters across the adult age spectrum

of a healthy European cohort and to analyze the associations between physical activity (PA) levels and CPET parameters; (5) To determine whether VO_2-kinetics is a useful marker for risk stratification of HF; and finally (6) to analyze whether a composite measure (health distance, HD) of physical fitness biomarkers can increase discriminative performance between healthy individuals and patients with HF compared to standard clinical biomarkers.

Methods

In this Ph.D. project, a literature review (publication 1) and one large project including two cross-sectional studies (publication 2-6) were conducted. In the literature review, five widely studied gas exchange variables from CPET (VO_{2peak}, VE/VCO_2 slope, exercise oscillatory ventilation (EOV), oxygen uptake efficiency slope (OUES) and $P_{ET}CO_2$) were evaluated regarding risk stratification and management of HFrEF based on nine different criteria (proof of concept, prospective validation, incremental value, clinical utility, clinical outcomes, cost-effectiveness, ease of use, methodological consensus and reference values). The literature search was performed for each of the five variables and the nine criteria separately, beginning with a search for systematic reviews using the Cochrane Library. Subsequently, simple search strings were used for electronic literature searches in the Medline and Embase databases.

The COmPLETE Project, the project of this Ph.D. Thesis, consists of two parts: COmPLETE-Health and COmPLETE-Heart (study protocol, publication 2). COmPLETE-Health examined physical fitness and CV markers in a healthy population aged between 20 and 90 years. A total number of approximately 490 participants with a valid cardiorespiratory fitness measurement (the primary outcome) were included. Participants were equally distributed over the 7 age decades from 20–30 to 80–90 and by sex (50% female). COmPLETE-Heart examined the same physical fitness and CV markers as COmPLETE-Health in 80 patients at different stages of chronic HF. CRF was assessed by CPET. Secondary outcomes included walking speed, balance, isometric strength, peak power, and handgrip strength. PA as a behavioral component was assessed objectively via accelerometry, and several CV assessments were performed.

For publication 3, CPET data from the COmPLETE-Health Study were analyzed involving 274 men and 252 women. All participants underwent a CPET until maximal voluntary exertion using a cycle ergometer. To determine new exhaustion criteria, based on maximal respiratory exchange ratio (RER_{max}) and age-predicted maximal heart rate (APMHR), one-sided lower tolerance intervals for the tests confirming VO_2 plateau status were calculated using a confidence level of 95% and a coverage of 90%.

In publication 4, CPET data of the COmPLETE-Health Study were further analyzed. Participant included in the final analysis, had to have reached secondary exhaustion criteria based on age-dependent RER cut-offs during the CPET. PA was objectively and continuously measured over 14 days using a triaxial accelerometer. Quantile curves were calculated for CPET parameters to

provide reference data. To investigate the associations between CPET parameters and PA levels, linear regression analysis was performed.

In publication 5, the CPET data of both the healthy participants of the COmPLETE-Health Study and the patients with HF of the COmPLETE-Heart Study were analyzed. To achieve comparability, we first created a matched dataset where we matched two healthy participants to every patient with HF according to age and sex (2:1 matching). The CPET was preceded by a 3-minute low-intensity warm-up and followed by a 3-minute recovery bout. VO_2-kinetics was calculated from the rest-to-exercise transition of the warm-up bout (on-kinetics), from the exercise-to-recovery transition following ramp test termination (off-kinetics), and from the initial delay of VO_2 during the warm-up to ramp-test transition (ramp-kinetics).

As in publication 5, both groups—COmPLETE-Health and COmPLETE-Heart—were analyzed in publication 6. Fifty-nine biomarkers in the categories fitness (cardiovascular endurance, muscle strength, and neuromuscular coordination) and general health (anthropometry, vascular and respiratory health and blood testing) were used for the analysis. HDs were computed for all relevant biomarkers and domain-specific subsets as the Mahalanobis distance defined for vectors of biomarker measurements that quantified the deviations of individuals' biomarker profiles from "optimal" values in the "reference population" (healthy study participants aged less than 40 years). Linear regressions were fitted with HD outcomes and disease status (HF/Healthy) and relevant covariates as predictors. Also were logistic regressions fitted for the disease status as the outcome and sex, age, and age^2 as covariates in the base model and the same covariates plus combinations of one or two HDs to compare different models' performance in predicting HF cases.

Results

Publication 1: The Role of Gas Exchange Variables in Cardiopulmonary Exercise Testing for Risk Stratification and Management of Heart Failure with Reduced Ejection Fraction (1).

This review demonstrated that the evidence supporting the clinical assessment of variables beyond peak VO_2 for HF patients with reduced ejection fraction is well established. The five variables: peak VO2 or predicted peak VO_2, VE/VCO_2 slope, EOV, OUES, and $P_{ET}CO_2$ provide evidence for the criteria "proof of concept", "prospective validation", and "incremental value". Based on the results of this review, the combined assessment of peak VO_2 or predicted peak VO_2, VE/VCO_2 slope, and EOV is recommended as part of the clinical examination. Further, because no additional costs are incurred in investigating the OUES and $P_{ET}CO_2$, these parameters should also be considered as part of routine analysis. Each of those five variables reflects in part a different pathophysiologic feature of HFrEF, and combining these variables delivers a broader three-dimensional picture of the

pathophysiologic process and severity of HFrEF. A multi-variable approach is, therefore, appropriate.

Publication 2: Functional Aging in Health and Heart Failure: the COmPLETE Study (2).

The COmPLETE study will allow for the investigation and characterization of the physical fitness components of endurance capacity, muscle strength, and neuromuscular coordination in individuals aged 20 to 90 years without chronic diseases and in patients with HF. The age-matched comparison with patients at different stages of HF may provide an estimate of the health distance of different fitness parameters to healthy individuals. Health distance provides a new complex measure of aging-related decline in the adaptive capacity of the organism by comparing the values of the physiological "norms" (COmPLETE-Health) with those with prevalent HF (our example). The study's results shall provide a better understanding of which functional characteristics should specifically be targeted in primary and secondary prevention to achieve optimal healthspan.

Publication 3: New Data-based Cutoffs for Maximal Exercise Criteria Across the Lifespan (3).

The analysis of 526 participants aged 20–90 years revealed data-based optimal secondary exhaustion criteria according to tolerance intervals optimizing the evaluation of VO_{2max}. These criteria are for the age group of 20 to 39 years: $RER_{max} \geq 1.13$, $APMHR_{210} \geq 96\%$, and $APMHR_{208}$ 93%; for the age group of 40 to 59 years: $RER_{max} \geq 1.10$, $APMHR_{210} \geq 99\%$, and $APMHR_{208}$ 92%; and, for the age group of 60 to 69 years: $RER_{max} \geq 1.06$, $APMHR_{210} \geq 99\%$, and $APMHR_{208}$ 89%. The proposed cut-off values for secondary criteria reduce the risk of underestimating VO_{2max}. The criteria differ clearly from previously used lower cut-offs and our results show that higher criteria need to be applied. Lower criteria than the ones we suggest would increase false-positive results, assuming participants are exhausted although, in fact, they are not.

Publication 4: Novel CPET Reference Values in Healthy Adults: Associations with Physical Activity (4).

VO_{2peak} values observed in the group aged 20–29 years were 46.6 ± 7.9 and 39.3 ± 6.5 (mL/kg/min) for males and females, respectively. On average, each age category (10-year increments) showed a 10% lower VO_{2peak} relative to the next younger age category. VO_{2peak} values of previous studies were, on average 7.5 (mL/kg/min) (20%) lower for males and 6.5 (mL/kg/min) (21%) lower for females. There was strong evidence supporting a positive association between VO_{2peak} (mL/kg/min) and the level of habitual PA performed at vigorous PA (estimate 0.26; $p < 0.001$].

Publication 5: VO_2 Kinetics: An Alternative to Peak VO_2 for Risk Stratification and Diagnosis in Heart Failure (5).

VO_2 off-kinetics demonstrated the highest z-score differences between healthy participants and patients with HF. Furthermore, off-kinetics were strongly associated with VO_{2peak}. In

contrast, ramp-kinetics and on-kinetics showed only minimal z-score differences between healthy participants and patients with HF. The best on- and off-kinetic parameters significantly improved a model to predict disease severity. However, there was no significant additional value of VO_2-kinetics when VO_{2peak} was part of the model.

Publication 6: Composite Measures of Physical Fitness to Discriminate between Healthy Aging and Heart Failure: the COmPLETE Study (6).

Nine out of ten calculated HDs showed evidence of group differences between the Healthy and the HF group ($p \leq 0.002$), and most models presented a negative estimate of the interaction term age by group ($p < 0.05$ for eight out of ten HDs). The predictive performance of HF cases of the base model significantly increased by adding HD *General health* or HD *Fitness* with an increase in the AUC estimate from 0.63 to 0.89 and 0.84, respectively. HD *Cardiovascular endurance* alone reached an AUC of 0.88. Further, there is evidence that the combination of HD *Cardiovascular endurance* and HD *General health* shows superior predictive power compared to when using HDs alone.

Conclusions

The results of this thesis provide further knowledge and advances in the assessment of physical fitness, particularly CPET markers for risk stratification and management of chronic diseases such as HF. According to our literature review: a multi-parameter approach including VO_{2peak}, VE/VCO_2 slope, and EOV is recommended when assessing HF patients with reduced ejection fraction by CPET. The application of the provided age-dependent exhaustion criteria reduces the risk of underestimating VO_{2peak} and provides a simple, yet effective tool. Further, reference values for maximal and several submaximal CPET parameters over a large age range are novel and differences to other studies are clinically highly relevant. Vigorous-intensity PA showed a strong positive association with higher VO_{2peak} and other performance-related CPET parameters within the healthy cohort. Further, VO_2-kinetics can provide an acceptable substitute if VO_{2peak} cannot be determined. VO_2 off-kinetics appears to be superior for distinguishing patients with HF and healthy participants compared to VO_2 on-kinetics and ramp-kinetics. Finally, when combining several physical fitness biomarkers, a significant difference in HD between healthy individuals and patients with HF can be observed. The application of HD could strengthen a comprehensive assessment of physical fitness and may present an optimal target for interventions to slow the decline of physical fitness with aging and, therefore, to increase healthspan.

Chapter 1

Background

Chapter 1 Background

1.1 Aging

Aging may become one of the most critical problems of our time. It is described as a problem that could develop to an equivalent scale as global warming—a “ticking time bomb” that could lead to a global healthcare crisis (7, 8).

Life expectancy at birth reached 81 years across the 28 EU member states in 2018 (9). For Switzerland, life expectancy for men corresponds to the European average of 81 years; for Swiss women, a higher life expectancy of 85 is reported (10). Moreover, life expectancy is projected to further increase in all industrialized countries in the next decades (11). Kontis et al. note that there is more than a 50% probability that by 2030, life expectancy for women in some industrialized countries will break the 90-year barrier (11). Such a level was deemed unattainable only 20 years ago. These projections show the continued increases in longevity.

These increases in life expectancy, coupled with lower birth rates, are leading to further changes in aging demographics. By the year 2050, the developed countries will have at least 25% of their population older than 65 years, with some regions exceeding 40% (7, 12). For Europe, this number will rise from 29.6% in 2016, to 51.2% in 2070, according to the 2018 European Commission Ageing Report (13). Whereas the number of people aged over 65 years is expected to double during this period, those aged 80 years or more will at least triple (7, 14).

The gain in life expectancy in recent decades have been mainly due to the prevention of early mortality, such as death around birth and death from infectious diseases, malnutrition, or accidents. Kontis et al., however, predict that a significant part of the projected gains in life expectancy at birth until 2030 will be due to enhanced longevity above the ages of 50 and 65 years (11). These longer lives are not automatically longer healthier lives, and the increase in life expectancy through modern medicine has its downsides. Age drives a set of disorders such as cancer, diabetes, kidney diseases, Alzheimer’s, Parkinson’s disease, stroke, cardiovascular disease, and resulting HF (15). These chronic age-related diseases are now the leading causes of morbidity and mortality in developed countries (16, 17). By far the largest risk factor of all these pathologies is aging (15, 18, 19).

It is important to note that aging is the driving factor of these disease and with increasing age, the vulnerability for diseases rise, although age is not a disease itself (20). Aging can be described by several characteristics according to Hayflick et. al. (20) and can, therefore, be clearly separated from age-associated disease: age changes 1) occur in every multicellular animal that reaches a fixed size at reproductive maturity, 2) cross nearly all species barriers, 3) occur only after the age of reproductive maturation, 3) occur in virtually all animate and inanimate matter, and 4) have the same universal molecular etiology; that is, thermodynamic instability. There is no disease or pathology that shares these characteristics of aging (20). It is important to note that this

dissertation will focus on age-related changes in physiological functions and age-related chronic disease rather than on research into the fundamental processes of aging itself.

The shift of the major causes of morbidity towards chronic disease and the changing aging demographics will lead to an epidemic of age-driven chronic disease. The total prevalence of people in Europe reporting heart or circulation problems in 2016 was 9.2% for both males and females (21). This figure will likely increase in the next 20 years to a similar scale as predicted for the US. Heidenreich et al. have recently estimated that by 2040, 40% of all adults in the U.S. will have at least one form of clinical cardiovascular disease (22). Other age-associated diseases such as Parkinson's disease or dementia, have comparable predictions (7, 23).

The consequences of the changing age demographics and the increasing prevalence of chronic diseases are multifaceted, including a reduced workforce, higher demand for health care and caregiving, increased pressure on health insurance programs and pension funds, changes in saving and consumption, housing and transport, and many other aspects of community life (8, 12, 15, 24).

Despite the increasing scale and certainty of the problem, also referred to as the "silver tsunami", no comprehensive plan has been developed to face these challenges (24). The contributions of physiology and preventive medicine, and the minimal contribution of the present thesis to that problem, will be described in the upcoming chapters.

1.1.1 Physiological Changes of Aging

The decline of physiological function with age presents a major obstacle to achieving optimal longevity and limiting the phase of morbidity. Declines of physiological functions that occur with advancing age have to be retarded in order to achieve optimal longevity with an increased healthspan (15, 24, 25). Physiological impairments will lead to functional limitations, increased risk of chronic diseases and disability, reduced productivity/independence/quality of life, and an increased risk of mortality (15, 26). Impaired physiological function of aging can have a negative impact on optimal longevity from several perspectives, with some of them being independent of a clinical disease. Dynapenia offers a highly suitable example. It involves the age-associated loss of muscle strength or power that is not caused by neurologic or muscular diseases (27). Factors discussed to drive this reduction are likely multifactorial and include subclinical deficits in the structure and function of the nervous system, impairments in the intrinsic force-generating properties of skeletal muscle, sarcopenia (age-related atrophy of muscle), and decreased PA (28-31). Dynapenia can have significant clinical consequences because it increases the risk for functional limitations, disability to perform basic activities of daily living, and mortality (31, 32).

A second example is the age-associated reduction of VO_{2max}. Age-associated reductions in maximal cardiac output of up to 30% are observed and are mainly due to a decrease in maximal heart rate (33). In addition to the reduction of maximal cardiac output, a reduction in peripheral oxygen

extraction leads to reduced maximal aerobic capacity (34, 35). Further age-associated factors, such as loss of muscle mass (sarcopenia) and muscle strength with age (dynapenia) (36), could be further contributing factors. From the behavioral components, a reduction in PA (in particular, vigorous PA) might be contributing to the decline in VO_{2max} with age (37, 38).

Changes such as a reduction in cardiorespiratory fitness or muscle strength impact functional status and quality of life, and can worsen into more fully developed medical disability with resulting further impairments; for example, through reduced PA.

A decline in physiological function is also an unwanted starting point or risk factor to chronic diseases of aging (15, 25). Several examples of reduced physiological function leading to increased risk of disease exist. Vascular dysfunction analyzed by endothelial function can be observed in otherwise apparently healthy older individuals and precedes clinical disease. Endothelial dysfunction seems to play a key role as a primary determinant of pathophysiology that leads to clinical atherosclerosis, hypertension, and stroke (18, 39). In 60% of asymptomatic individuals aged 68.9 ± 6.0 years, subclinical atherosclerosis was detectable in the carotid and coronary arteries (40). Further, several cardiac structural and functional alterations can be observed with age in healthy humans. One of these alterations is an impaired diastolic filling of the left ventricle that is closely related to the steep increases in chronic HF cases seen with increasing age (33). Often, the declines in physiological function lead to chronic disease, and the disease in turn further reduces function—a vicious cycle can develop. Endothelial dysfunction is a particularly good example. A single physiological dysfunction can result in an increased risk of clinical pathologies of various types. Arterial stiffness of the large elastic arteries driven by age, a hallmark of vascular aging (18), has been linked to several chronic disorders of advancing age, including systolic hypertension, coronary artery disease, stroke, cognitive impairment, Alzheimer's Disease, chronic kidney disease and even motor dysfunction, including falls (41-43).

Declines in physiological function are not only risk factors for chronic diseases but also independent predictors of mortality (15). Cardiorespiratory fitness, for example, is a particularly strong predictor of survival independent of other risk factors (44-46). Other markers of physical fitness/functional capacity, such as gait speed (47, 48), grip strength (49), and whole-body strength including maximal measures for leg and bench presses (50), are also all strong predictors of survival in middle age to older healthy adults.

To summarize, with age, a decline in physiological function can be observed. The larger these declines are, the more likely it is that functional status is reduced and the risk of morbidity, disability, and mortality is increased. Therefore, age-associated decreases in physiological function should be evaluated in clinical practice and then used as a critical target for primary and secondary prevention. Specific and individualized targeting of physiological functional decline could present an opportunity to handle the approaching pandemic of chronic disease and disability caused by the changes in age demographics of developed and developing countries (15, 24, 25).

1.1.2 Optimal Aging vs. Early Aging and Onset of Chronic Diseases

Although medical advances have resulted in increased mean lifespan, as described in 1.1., it has been suggested that this increase is primarily due to people surviving longer with age-associated disease and disability and does not necessarily equate to an increased time without any clinical disease (51, 52). A concept to counteract the traditional way of thinking of age-associated chronic diseases was introduced as early as 1980 (53) and recently further developed by leaders in the field of biomedical aging research (25, 54). This concept's goal is to increase the years free of disease and to shorten the time period with a disease during the final part of one's life. Taking into account the aging of the organism and considering the decline of physiological function and functional limitations that may occur before the onset of a disease, the term "healthspan" was recently introduced (25, 51, 54). Healthspan is defined as a period of relatively healthy aging free of major chronic clinical disease and disability. According to this concept, life presents two phases: a phase of relatively healthy aging (healthspan) and a phase of age-associated chronic disease and disability. During the period of relatively healthy aging, various physiological functions decline, including the CV system or physical fitness. The age-related decline in functions of the organ systems increase the risk of chronic diseases. Curative medicine prolongs a lifespan but may also be associated with a longer time of disability. Optimal healthspan and the compression of morbidity can be attained by optimal prevention and strategies to maintain physiological function into old age (15, 53).

One aspect of physiological function undergoing a decline with increasing age is physical fitness. The following chapters will focus on the age-related decline of physical fitness components, norm values of chosen physical fitness variables, the challenges of the measurement of these variables, and differences of physical fitness components between healthy individuals and patients with chronic HF, a classic age-associated chronic disease.

1.1.3 Age-related Decline of Physical Fitness Components

Because there is no single agreed-upon definition of fitness in the scientific literature, some of the definitions often used are provided in the following. The American College of Sports Medicine's (ACSM) "Guidelines for Exercise Testing and Prescription" define fitness as "a set of attributes or characteristics that people have or achieve that relates to the ability to perform PA" (55) . Another source published in Medicine and Science in Sports and Exercise (56) defines fitness as "the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy [leisure] pursuits and to meet unforeseen emergencies". Several research groups also use the term "fitness" when describing cardiorespiratory fitness, a measure or estimate of VO_{2max} and therefore evaluating aerobic capacity. Cardiorespiratory fitness is one of several components of physical fitness but not a suitable description or measure of overall physical fitness. Further physical fitness was operationalized as: "[a set of] measurable health and skill-related attributes" that include cardiorespiratory fitness, muscular strength and endurance, body composition and

flexibility, balance, agility, reaction time, and power (57). In this project and dissertation, physical fitness is simplified to the following three major components: cardiovascular endurance, muscle strength/power, and neuromuscular coordination. These three major components of physical fitness have shown predictive and concurrent validity with health and performance outcomes and will be discussed in the following section (58).

The deterioration of the main components of physical fitness (i.e., cardiovascular endurance, muscle strength/power, and neuromuscular coordination) due to inactivity or insufficient PA is associated with lower capacities of the CV system, skeletal muscles, and the neuromuscular system (34, 59). The final state of the unfavorable decline of physical fitness with age or due to diseases is generally regarded as frailty or disability (60).

Maximal Oxygen Consumption

Maximal oxygen consumption (VO_{2max}) describes the upper limit of maximal energy production through oxidative phosphorylation and is generally considered to be the gold standard for measurement of aerobic fitness and a primary determinant of endurance exercise performance. According to cross-sectional studies, VO_{2max} declines on average by approximately 10% per decade after the age of 20–30 years (35, 61, 62). A longitudinal study showed, however, that the rate of decline in VO_{2peak} in healthy adults is non-linear, with declines from 3–6% per decade in the 20s and 30s and around 20% per decade in those aged 70 years and older (34). The Fick equation is the exact physiological definition of VO_{2max}: maximal cardiac output × maximal arterio-venous O_2 difference. The determinants contributing to the decline in VO_{2max} are not yet fully understood; however, research with master athletes provides insight into the decline of physiological function affecting VO_{2max} with aging in the absence of chronic disease. A reduction in both maximal cardiac output and maximal arterio-venous O_2 difference plays a role in this process. A large evidence base exists describing a decrease of cardiac output with advancing age in healthy sedentary and highly active adults (63), (64, 65). A major component of the reduction in cardiac output is the age-related decline in maximum heart rate at a rate of ∼0.7 bpm per year in healthy sedentary, recreationally active, and endurance exercise-trained adults (66). Discussed mechanisms include a slower conduction velocity, reduced responsiveness of the sinoatrial node to β-adrenergic stimulation, and decreased intrinsic heart rate (67). Maximal stroke volume seems to be slightly reduced compared to young individuals (64). There is, however, only rather limited evidence explaining the determinants of these small changes in maximal stroke volume. Total blood volume appears to be preserved in older endurance-trained individuals (68). Other suggested contributors to the reduction in cardiac output are determinants of the cardiac preload, including LV end-diastolic pressure, diastolic filling time, venomotor tone, myocardial compliance, and/or a combination of these factors (69, 70). Large elastic arterial stiffening with advancing age leads to an increased aortic impedance and vascular afterload,

thereby reduce the ejection of blood from the left ventricle during systole and, consequently, stroke volume during exercise (71). Maximal arterio-venous O_2 difference describes the capacity of the working skeletal muscles and also the respiratory muscle to extract and utilize oxygen for energy production. A modest reduction of 5–10% of maximal arterio-venous O_2 difference with increasing age is observed in healthy trained individuals (65) (35) and correlates with reductions in capillary density and mitochondrial enzyme activities in individuals of advanced age (72). Several pieces of evidence indicate that the reduction is more likely due to the oxygen delivery rather than oxygen extraction in the muscle and lungs (72, 73). A relationship between skeletal muscle mass and maximal aerobic capacity among healthy individuals has been observed (74, 75). Reduced skeletal muscle mass could impact maximal arterio-venous O_2 difference, but could also influence VO_{2max} negatively via central circulatory function involving blood volume, stroke volume, and cardiac output (63).

Muscle Mass, Strength, and Power

Muscle atrophy during aging, termed “sarcopenia”, has been described in cross-sectional studies to be approximately 27% between the ages of 18 and 88 years (76). Further, it has been shown that muscles of the lower body undergo increased age-related atrophy compared to the muscles of the upper body (76, 77). Longitudinal data suggest that the rate of the loss in muscle mass is even higher than once described in cross-sectional studies with an approximate reduction of 1% per year (78-80). Underlying physiological determinants could be a decrease in number and size of type II fibers (81, 82) and an increase in the proportion of smaller slower oxidative type I muscle fibers (type I) (83, 84).

The decline of muscle strength with age, referred to as “dynapenia” (30), exceeds the loss of muscle mass, as shown in both cross-sectional and longitudinal studies (79, 85, 86). These results indicate that a reduction in “muscle quality” (force per unit muscle mass) can be observed, describing a decrease in the ability of the remaining muscle tissue to generate force and power. Discussed determinants of this reductions are an increase in coactivation of antagonist muscles that increases with age, an increase in muscle fat infiltration, and reduced levels of voluntary activation (87).

The decline in power-generating capacity (i.e. rate of force development (RFD, muscle power)) observed with advancing age is even more pronounced compared to the decline in force generating capacity (88-90), with some estimates of the decline in power as high as 35% per decade after the age of 65 years (91). The reduction in muscle power with age is linked to reductions in the force-generating capacity and a decrease in the maximum shortening velocity (92, 93). The atrophy and loss of type II muscle fibers are likely components of the reduction in the maximal velocity of shortening (94).

Neuromuscular Coordination

Balance, also described as postural control, is a complex motor skill derived from the interaction of multiple sensorimotor processes. The two major goals of balance are postural orientation and postural equilibrium (95). Both balance and gait performance show a decline with age (96, 97). Several cross-sectional studies have demonstrated that the deterioration of balance performance begins relatively early in adulthood (30–39 years) and decreases linearly (98, 99). Further, when analyzing balance performance and gait speed combined with a cognitive (i.e., serial subtractions by three) and/or a motor interference/task, elderly individuals showed a decreased performance compared to their younger counterparts (100). Declines in gait and balance performance in older adults are a consequence of underlying changes in peripheral systems (101) but are also associated with smaller sensorimotor regions within the motor, visuospatial, and cognitive speed domains and, therefore, selective changes in brain structure (102).

1.2 Heart Failure as an Age-Related Chronic Disease

Heart failure is a form of cardiovascular disorder. Specifically, it is described as a complex clinical syndrome rather than a disease and is characterized by the reduced ability of the heart to pump and/or to fill with blood (103-106). In the context of the present dissertation, we are primarily interested in the physiological aspect of HF; therefore, the following definition of HF will be used: "an inadequate cardiac output to meet metabolic demands or adequate cardiac output secondary to compensatory neurohormonal activation (generally manifesting as increased left ventricular filling pressure)" (104). Or simplified in lay terms: the heart is unable to "pump" sufficiently to maintain blood flow to meet the body's needs. HF has recently been subclassified into three subtypes according to the ejection fraction, natriuretic peptide levels, and the presence of structural heart disease and diastolic dysfunction, namely HF with reduced ejection fraction (HFrEF), HF with preserved ejection fraction (HFpEF), and HF with mid-range ejection fraction (HFmrEF) (107).

1.2.1 Prevalence, Incidence, and Risk

Cardiovascular diseases, which include HF, are the leading cause of death in the EU (9) and worldwide (108). Between 2007 and 2017, an increase of 21% in deaths attributed to CVD was reported with 17.8 million deaths in 2017 globally. By 2030, CVD is expected to account for >23.6 million deaths per year (109). Data from the World Economic Forum found that CVD represented approximately half of all non-communicable disease deaths worldwide (110). The prevalence of HF specifically continues to rise over time in sync with the aging population. HF has been defined as a global pandemic based on the large number of people affected by the syndrome worldwide (111). In the US, an estimated 6.2 million individuals suffered from HF syndrome between 2013 and 2016 (16). Although a shortage of data on HF incidence and prevalence exists for Switzerland,

based on the data of other European countries, 13,000 new cases per year have been estimated (112). Lifetime risk for HF in the U.S. population at the ages of 45 through 95 years is reported to be between 20–46% (113). Using a dichotomous classification, approximately half of hospitalized HF events are characterized by HFrEF, and the other half by HFpEF (114). Both the prevalence of HFpEF and HFrEF appears to increase over time along with the changing age demographics. HFpEF, however, could be more dominant in driving the increasing prevalence of HF (16, 115). Based on a large European HF registry, initiated by the European Society of Cardiology, the 1-year mortality rate for HF was estimated to be 6.4%, and the rate for the combined endpoint of mortality or hospitalization within 1 year was 14.5 % (116). Further, HF is characterized by a lower quality of life compared to many other chronic diseases (117), with similar results observed in patients with HFpEF and HFrEF (118). A Scandinavian study confirmed the major health burden of HF and reports nearly identical rates of premature life-years lost due to HF and of those lost due to the most common forms of cancer (119). A recent study showed that an ideal score in the American Heart Association's (AHA) "Life's Simple 7" (optimal profiles in BMI, PA, diet, cholesterol, blood pressure, glucose, and not smoking) (16), was associated with a 55% lower risk for HF compared with a low, suboptimal score. Preventive strategies that target the Life's Simple 7 risk factors could, therefore, have a significant impact on lowering HF incidence in the population (16). Most of Life's Simple 7 risk factors are largely lifestyle behavior related. Health care cost related to HF are >10% of the total expenditure for CVDs and are projected to increase by 127% between 2012 and 2030 in the United States (120). Comparable cost developments can be expected in European countries with their high percentage of old individuals. In summary, the high morbidity, mortality, and associated impairments caused by HF make this chronic condition a significant health care concern (16).

1.2.2 Diagnosis, Prognosis, and Risk Stratification

According to the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic HF, the diagnostic algorithm of HF includes assessing clinical history, performing a physical examination, and investigating whether an abnormal electrocardiogram is present (121). If one of the above is positive, natriuretic peptides (N-terminal pro-BNP, NT-proBNP; or B-type natriuretic peptide, BNP) levels are determined and interpreted using the cutoff values of B-type natriuretic peptide ≥35 pg/mL and for N-terminal pro-BNP (NT-proBNP) ≥125 pg/mL (121). Even though values are lower for HFpEF than for HFrEF on average, the same diagnostic values apply (122). To establish a HF diagnosis, echocardiography is applied and serves as a useful and widely available test in patients with suspected HF. Echocardiographic evaluation provides instant information regarding ventricular systolic and diastolic function, chamber volumes, wall thickness, valve function, and pulmonary hypertension (121, 123, 124). All of the above steps are crucial in establishing an initial diagnosis and in determining appropriate treatment (121).

Accurate risk assessment methods and prognostic variables for morbidity, disability, and death for HF patients are essential to guide clinical decisions for therapeutic strategies with the ultimate goal of decreasing risk and improving health outcomes. Accurate determination of HF progression presents a challenge because it is influenced by many factors. Numerous prognostic markers of death and/or hospitalization have been identified in patients with HF. These markers can be categorized as: clinical status, demographic data, severity of HF, myocardial remodeling and severity of heart dysfunction, biomarkers of neurohormonal activation, co-morbidities, and genetic testing (121). Of the listed categories, severity of HF appears to be most essential for risk stratification and prognosis. Reduced exercise capacity and, therefore, cardiorespiratory fitness is the hallmark symptom of HF. Traditionally, limitations during PA have been assessed in HF patients by New York Heart Association (NYHA) class. Even though NYHA class serves as an initial indicator of functional limitations, it is both subjective and insensitive (125). The 6-minute walk test presents a more objective method compared to the NYHA functional class but still can be heavily influenced by the patient's motivation. Further, the tests provide no objective data to judge the patient's level of exhaustion (125). Gold standard assessment of CRF is performed by the measurement of VO_{2peak} by cardiopulmonary exercise testing (CPET). Due to the large evidence base, CPET is a well-recognized tool and offers a valuable and accurate method for risk stratification in HF (125, 126). Several CPET variables (127-135) and their combination (136, 137) have been identified as predictors of HF prognosis. Further VO_{2peak} is applied as heart transplant criteria and to optimize its timing (138, 139). Although research on CPET parameters and HF prognosis has been highly promising, risk stratification with CPET parameters is not yet integrated into clinical practice regularly. A combination of demographic data, medical history, laboratory values, HF treatment background, and CPET variables seems highly promising (140, 141). CPET parameters and their value in the clinical setting for risk stratification and the management of HF are discussed in detail in the following chapter.

1.2.3 Physical Fitness Components in Heart Failure

Cardiorespiratory Fitness and Physical Activity

Before exploring the relationship of exercise and CRF to HF, it is important to distinguish between PA and fitness. Although closely related, they represent distinct characteristics (57). Physical activity is any bodily movement produced by skeletal muscle that results in energy expenditure. CRF is either an estimate or a direct measure (then used as a synonym of VO_{2peak}) and the primary characteristics of aerobic endurance capacity (125). Improvement in CRF can be achieved by increasing one's PA.

In recent decades, there has been increasing recognition that higher levels of both CRF and PA are associated with improved health outcomes across a broad range of CV disease (142-144), including HF (145-147). CRF was, therefore, encouraged as an important health marker in apparently healthy individuals and HF patients and described as a "clinical vital sign" by professional

organizations (126, 148). Among 20,642 subjects, the Copper Center Longitudinal Study (149) reported for the subjects classifying in the lowest fitness quartile during mid-life a 3–4-fold higher rate of hospitalization for HF later in life compared to the highest quartile. This observation emphasizes the importance of CRF levels during midlife. Further, Myers et al. (150) demonstrated that every additional 1-MET (metabolic equivalent of task) in CRF was associated with a 19% reduction in risk for developing HF. When comparing subjects in the least-fit quintile with subjects classifying in the highest quintile according to CRF levels, risk for developing HF was 76% higher (150). A follow-up study over a mean of 21 years reported that every 1-MET increment in CRF was associated with a 21% multivariable-adjusted risk of HF (151). Similarly, a meta-analysis reported a random-effects model estimate of 21% lower risk of HF for every additional 1-MET higher CRF (147). Based on these results, it could be argued that through an intervention targeting CRF at midlife, HF risk can be favorably modified.

Exercise training and PA are the primary intervention strategies to increase CRF and, therefore, HF outcomes. A large exercise intervention trial with HF patients reported that a modest increase in CRF over the 3-months intervention period was associated with a lower risk of all-cause mortality and hospitalization (152). Beyond the impact on CRF and hard endpoints, PA also has favorable influences on the classic HF risk factors, including hypertension and diabetes mellitus (148, 153). Even minimal changes (1 MET; 3.5mL/kg/min) in CRF can have a beneficial impact on HF incidence (150). Typically, increases in CRF of approximately 2–7 mL/kg/min (1–2 MET) are observed by standard exercise intervention programs (126, 148). Improvements in CRF through PA promotions can play a potentially important role in the prevention of HF (149, 154, 155). Prevention strategies for HF other than the control of traditional risk factors, particularly blood pressure, are relatively limited (121). In particular, the prevention and treatment of HFpEF remains a challenge. The result of the Framingham Heart Study found that the protective effect of PA on HFrEF patients could be extended to patients with HFpEF (156). The protective effect of PA and CRF on HF incidence is thought to be partly direct and partly due to a positive effect of PA on risk factors (157). Individuals with higher PA levels and higher CRF values compared to their counterparts show less HF risk factors (158, 159). The muted increase in blood pressure with increasing age in fitter and more active individuals is certainly to be emphasized as a potential mechanism (160). Further physiological benefits of PA include improved insulin resistance, better lipid profiles, and reduced obesity (153, 161). In addition, endothelial function has been shown to improve after exercise training interventions and could contribute to the protective effect (162). Further, left ventricular compliance can be positively affected by PA (69). The Cooper Center Longitudinal Study showed that a low CRF was associated with a higher prevalence of concentric remodeling and diastolic dysfunction. PA, therefore, may lower HF risk through its effect on favorable cardiac remodeling and improved diastolic function (163). The demonstration of the inverse association between fitness and the development of HF, and the potential to increase CRF in HF by exercise interventions, does provide clinicians with further evidence to target CRF and encourage patients to increase their PA levels.

Additional Physical Fitness Parameters

The determinants and process by which CRF is limited in HF are still not fully understood, particularly for HFpEF (164). The association between CRF and central hemodynamics is relatively small (164-166). A recent study by Houstis et al. showed that 27% of improvements in VO_{2peak} are determined by the muscle oxygen diffusion capacity and only about 7% by the cardiac output (167). Peripheral determinants of exercise capacity seem, therefore, of importance to assess and target. Peripheral differences within the skeletal musculature between healthy and HF subjects have been observed (168-173). A large evidence base suggests that in both HFpEF and HFrEF patients, non-cardiac factors contribute to reduced VO_{2peak} levels. These non-cardiac factors may, therefore, also be major contributors to the improvement in VO_{2peak} after endurance exercise training (169, 174-177). A reduced percentage of type I (oxidative) muscle fibers and a decreased number of capillaries per fiber are thought to contribute significantly to the reduced CRF of HF patients (178-180). Further, muscle mass is known to be reduced in chronic HFrEF by approximately 20% and in HFpEF by approximately 10%, respectively (181, 182), and muscle wasting and sarcopenia can predict mortality in HF patients (183). Although a shift of type I to type II muscle fibers can be observed in HF patients, strength performance decreases significantly with increased disease severity (182). HF patients show reduced quadriceps maximal isometric strength as well as reduced hand grip strength (182, 184, 185). Both quantitative and qualitative skeletal muscle abnormalities can explain these reductions (185). Increasing muscle weakness is associated with an increasing exercise limitation and can become a major determinant of reduced exercise capacity (185). The reduced muscle mass seems only in part able to explain the lower endurance and strength capacity in HF, however (171, 186). Beyond the physiological changes described above, reductions in mitochondrial content, cytochrome oxidase activity, and succinate dehydrogenase might contribute to the exercise limitations in HF patients (187, 188). A strong correlation between CRF and local exercise performance (muscle strength) has been observed (189). Further, an interrelationship between muscle strength or fatigue and muscle wasting (186) and TNF-α (190) has been described. These findings could explain the strong association of muscle strength and HF outcome (189). The described studies above indicate that a simultaneous measurement of CRF, muscle mass and muscle function could provide insights into the process of functional decline in HF patients without invasive investigations.

In addition to strength performance, measures of overall frailty have gained attention in the field of cardiology because HF primarily affects older individuals (16). Several studies showed that patients with HF have a higher prevalence of frailty (191-193). Frailty is clinically recognized as a syndrome of loss of reserves that enhances vulnerability, thus increasing the risk of major events and disability in patients with or without HF (194). Frailty reflects declining physiological function and may explain the heterogeneity in clinical outcomes within older individuals and HF patients. Considering the impaired muscle mass, strength, and CRF in the HF population, the high prevalence of frailty is not a surprise. Studies investigating the prognostic role of frailty in patients with HF found that frailty is a predictive marker of death and hospitalization (193, 195, 196). The

assessment of gait speed has been demonstrated to be a reliable single marker of frailty in older HF patients; it is independently associated with death, hospitalization for HF, and all-cause hospitalization and it improves risk stratification (197). Assessment of frailty using gait speed is simple and might provide important information in the clinical evaluation of HF patients with advanced age.

1.3 Physical Fitness Testing

1.3.1 Cardiopulmonary Exercise Testing (CPET)

A variety of maximal and submaximal exercise tests have been used to assess cardiorespiratory fitness and exercise tolerance, or to predict all-cause mortality in healthy and patient populations (126). However, only the evaluation of maximum oxygen uptake (VO_{2max}) achieved during severe-intensity exercise and exercise involving large muscle mass (i.e. running or cycling) actually assesses the upper limit of the oxygen transport and utilization system. VO_{2max} measures the integrated functioning of the pulmonary, cardiovascular, and muscle systems to uptake, transport, and utilize O_2 predominantly in the mitochondria of the contracting muscles. The initial observation of VO_{2max} was described already in 1923 by Hill and Lupton (198). They discovered that an upper limit of maximal oxygen transport exists from the environment to the working muscles, supporting oxidative production of ATP to perform physical work. Routinely, VO_{2max} is assessed by CPET. CPET describes a test in which either speed or work rate progressively increase up to the maximal physical exhaustion of the subject, with an assessment of gas exchange. Ideally, a ramp protocol over the course of 6–18 min is used (199, 200). Prior to the introduction of breath-by-breath gas exchange measurements, using rapidly responding gas analyzers, VO_{2max} was measured most commonly using Douglas bags and a discontinuous series of progressively higher constant-work rate intervals. Every test was performed until a quasi-steady state (if achievable) was reached or until maximal exhaustion intervened. Thanks to the advancement in analysis methods providing continuous breath-by breath analysis, concomitant assessment of oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) can be determined. CPET and the measurement of these three gas exchange parameters, their relations to each other and to the workload, and their combination with standard variables of clinical exercise testing (i.e. heart rate, blood pressure response, and electrocardiography findings) provide a large amount of crucial information (125). Therefore, CPET has several advancements beyond other CRF assessment methods, specifically in the clinical setting where it provides a wide array of unique

and clinically useful incremental forms of information and gives insight into the underlining pathologies of exercise limitations. In PubMed, over 10,000 articles are listed with the search term "VO_{2max}" and over 3,000 with the term "VO_{2peak}". This observation underlines the importance and relevance of measuring maximal oxygen uptake for understanding physiological function and exercise capacity in health and disease. The assessment of VO_{2peak} and other CPET variables have substantial clinical utility for measuring and understanding physiological dysfunction in aging. Further, CPET can provide important information for several pathological conditions that impact pulmonary, cardiovascular, and muscle systems, including chronic HF, COPD, diabetes, and HIV-AIDS. Moreover, the power of VO_{2peak} to noninvasively determine the efficacy of interventions such as exercise training, pharmaceuticals, or other ergogenic strategies in health and in disease conditions is substantial. In recognition of the importance of CRF to one's current and future health, particularly when CRF is assessed by CPET, the AHA recently published a scientific statement affording vital sign status to this important health measure (126).

1.3.2 Limitations and Challenges of CPET

CPET, as is the case for any other diagnostic or prognostic measure, offers some challenges. Several advancements have been made in CPET research and practice in recent years, such as technical development of the equipment and software, agreement on suitable protocols, and a wider application of the test. Two of the remaining challenges include: 1) the evaluation of whether the true individual physiological limit is reached and VO_{2max} can be determined, or if the VO_{2peak} measured is at least close to the potential maximum and 2) accurate and suitable normative reference values of healthy individuals across lifespan, as well as thresholds that denote varying degrees of health and risk for future adverse events for various CPET parameters. These two topics are discussed in the next two chapters and covered in two of the included publications in this thesis.

Exhaustion

Because VO_{2peak}/VO_{2max} is used as a crucial outcome, including 1) as a primary outcome in randomized controlled trials, 2) as a clinical vital sign, to stratify risk, or to guide therapeutic strategies, and 3) as a criterion for clinical decision-making (e.g. for heart transplantation), accurate and reliable determination of the parameter is essential. Achieving physical exhaustion and a pre-defined physiological limit is necessary to determine VO_{2max}/VO_{2peak} but making the distinction between those participants who have reached this limit and those who have not remains a challenge. Similar to any other exercise test, the subject's motivation and or naivety can limit the quality of the outcome. To maximize the signal-to-noise ratio, it is crucial to measure VO_{2max}/VO_{2peak} with sufficient rigor. Therefore, a reliable determination of an individual's physiological limits is important. Compared to other exercise test protocols, such as time trials,

familiarization does not seem to be essential for achieving a valid outcome in a CPET using a ramp protocol (201). Further, in contrast to other exercise tests evaluating CRF (i.e. the 1-mile walk test, Cooper test, or the 6-min walk test (202)), CPET provides objective quantitative information about the subject's level of effort and exhaustion. Further, it is important to note that VO_{2peak} is not the only variable of interest when performing a CPET; several useful submaximal parameters have been shown to deliver important diagnostic and prognostic information on a subject's health status and are independent of the subject's level of exhaustion (see chapter 1.3.3). The criterion to distinguish between VO_{2peak} and VO_{2max} is the occurrence of a VO_2 plateau. This criterion, however, has several limitations. First, it requires relatively complex data analyses. Second, numerous definitions have been proposed, leading to a substantial amount of controversy (203). Third, approximately 50% of healthy subjects fail to produce a VO_2 plateau even when the actual VO_{2max} is, in fact, achieved (204-208); this proportion can be even higher in the elderly, in unfit individuals, and in patients with CV or pulmonary disease (209). Even when an appropriate VO_2 plateau analysis is performed, for the subjects not confirming VO_2 plateau status, another option must be applied.

Recently, verification tests have been proposed as a relatively new method to evaluate whether an individual's physiological limit has been reached. These tests have their origin in the field of performance sports but are now also proposed in the clinical setting (210). The goal of a verification test is to provoke a VO_2 plateau by inducing a supramaximal load (i.e., verification-phase) following a regular ramp protocol. The exact protocols to perform these tests are, however, not yet standardized. Different verification protocols vary widely in duration and workload of regeneration phase, increase of workload during the verification-phase, and most importantly, maximum workload during the verification-phase (204, 205, 211-213). Poole and Jones proposed the use of one verification bout at 110% peak work rate (210); several authors, however, use a single or several bouts at a lower intensity (204, 205, 213), whereas others use a higher intensity (211, 214). Although some research groups draw conclusions of their study results that support the use of the verification tests, others describe their limitations generally and specifically in the clinical setting (201). Further, many studies that promote the use of verification tests failed to report the required data to support the use of verification phases. In detail, it has been criticized that VO_2 from the initial ramp phase and VO_2 from the verification phase were only compared on a group level, and it was not reported in which proportion of participants VO_{2max} could actually be verified (201, 215). In addition, it remains unknown what should be done when a plateau is *not* evident, particularly in cases when the VO_{2peak} measured during the ramp test is higher than the values observed during verification bouts. The feasibility of verification tests in the clinical setting must be questioned because they are highly time consuming and add to the financial cost of clinical testing. Two recent studies also showed that these tests offer only limited or no benefit (216, 217).

In those participants who show no VO_2 plateau and without a verified VO_{2max}, secondary criteria remain the third option to judge someone's exhaustion level (203, 210, 215). As their name already implies (secondary), these criteria will not be able to demonstrate with certainty whether or not VO_{2max} was reached. However, secondary criteria might have the potential to increase precision in the VO_{2peak} evaluation when chosen appropriately. The use of the term VO_{2peak} instead of VO_{2max} does not solve the issue of a subject's effort and a submaximal test (201). Whether the term VO_{2max} or VO_{2peak} is used, the goal should always be that the measured value comes as close to the true VO_{2max} as possible, even if it cannot be confirmed by a plateau or further tests. To achieve this goal, secondary criteria might be helpful; however, such criteria are rarely reported. According to Midgley et al., only 76% of studies published in "Medicine and Science in Sports and Exercise" from October 1993 to May 1994 (209) and 44% of studies published from October 2005 to May 2006 (203) reported secondary criteria for VO_{2max}. The most common secondary VO_{2max} criteria are maximum respiratory exchange ratio (RER_{max}), maximum heart rate (HR_{max}), maximum rating of perceived exertion (RPE_{max}), and maximum concentration of blood lactate (BL_{max}). Cutoff values for these parameters at which maximal physiologic effort is accepted for middle-aged participants vary widely, including RER values >1.00 (218), >1.05 and >1.1 ; 85% (34) to 100% of the age-predicted HR_{max}; ≥ 17 to ≥ 19 RPE_{max}; and from ≥ 4 to ≥ 10 mmol/L for BL_{max} (203, 209, 219-221). Knaier et al. (215) and Midgley et al. (203) noted that participants reached these criteria in nearly all studies, leaving room for speculation as to whether exhaustion criteria were defined post-analysis. Clearly, criteria should be determined in the study protocol and before the tests. Depending on the setting and the indication of the test, and the subjects tested, different secondary criteria and/or cutoffs might be appropriate. RER, HR, or BL can show some high variations between different groups. A "one-size-fits-all" approach would likely not improve the accuracy of VO_{2peak} determination but rather lead to a high number of type I and type II errors. In particular, blood lactate concentration has been shown to vary from approximately 2 to 17 mmol/L (222) in maximal tests. HR_{max} or RER_{max} can also vary widely between different fitness levels and age groups (215, 220). Further, it should be considered that some secondary criteria might be more useful than others in distinct populations. For instance, when testing patients taking β-blockers, HR_{max} would not be appropriate as a maximal exercise criterion. This population will experience bradycardia during exercise and evince very low HR_{max} values, whether or not their individual physiological limit of oxygen uptake is reached (210). The exercise protocol used also influences secondary criteria such as HR, BL_{max}, or RER (223, 224). Therefore, different cutoffs for secondary exhaustion criteria for different populations, age groups, and exercise protocols might have to be applied. Further research is required on this topic. Choosing secondary criteria for maximal exercise testing is always a trade-off between mistakenly assuming that subjects have reached VO_{2max} when they have not (i.e., low criteria, type I error) and declaring subjects to have not reached VO_{2max} even though they have (i.e., high criteria, type II error). Current, data-based cutoffs are only available for athletes (215) but not for the general healthy population or for different disease populations. Available recommendations are either based on expert opinions or

on inappropriate data analysis and their conclusions (220). Therefore, aim 3 of this thesis was to address this research gap.

Reference Values

Similar to all other clinical variables, CPET variables only have value when they can be compared to the values that are outside the usual spread of values found in health or within the spread of values typically found in disease (225). Therefore, reference values are established for surrogate markers and used to interpret an individual's test results. Reference values are also one of the nine essential criteria defined by the AHA (226) and Vlachopolus et al. (227) to be fulfilled by a surrogate marker. Reference values for a given test or parameter are based on the results that are observed in apparently healthy individuals (225). Only when we are aware of the normal, physiological value of CPET variables, can the distinction between a physiological from a pathological response to an exercise test be made. When reviewing the results of an exercise test, an individual's CPET variables (i.e. VO_{2peak}) should initially be considered in terms of what is "normal" for a given individual if he or she were healthy. This consideration is critical because CRF decreases with age, and higher values are generally observed in men compared to women. Thus, a VO_{2peak} of 30 ml/kg/min needs to be interpreted differently for an 80-year-old woman than a 30-year-old man. Knowing an individual's exercise capacity relative to their peers will not only help to optimize risk stratification but can also facilitate communication between health care professionals and patients regarding health risks. Further, it will provide a baseline for improving CRF, and provide support for the use of exercise as medicine (228).

In 2003, the statement on CPET by the American Thoracic Society and the American College of Chest Physicians recognized that having normal reference values "is critical to any interpretative scheme of CPET variables" (229). However, they write in their report that at the time, no optimal set of reference values existed. In 2019, Takken et al. (230) performed an updated systematic review of the literature on reference values for CPET and noted that only 4 studies met their criteria for high quality (230) of which 2 were from the same data base. Compared to the review published in 2014 (231), more data have been published in the last five years compared to the 35 years before, indicating that the need for CPET reference data has been recognized. However, the review further describes that there is still a lot of progress to be made. Quality can be further improved by performing a sample size calculation, a good quality assurance of equipment and methodologies, and by the exclusion of risk factors of the sample studied, such as smoking that can significantly influenc CPET parameters. The authors further recommend to improve the quality of CPET norm values by measuring and reporting the level of PA in the sample studied (230). Physical activity is the major behavioral component affecting VO_{2peak} and other CPET markers and remains the main method to increase $VO_{2peak.}$ A lack of reference data of subjects older than 70 years is apparent (230). With the increasing number of individuals aged between 70–90 years undergoing CPET, there is a need for reference values for apparently healthy individuals in this age group.

The AHA has recognized the clear need for developing norm values for VO_{2peak} and formed an advisory board and an initiative (FRIEND, Fitness Registry and the Importance of Exercise National Database) for a U.S. database for CRF norm values (232). The overreaching goal of FRIEND is to enhance the quality of CRF assessment, particularly through widespread use of CPET, and to promote CRF via physical activity in the United States. In recent years, FRIEND has published large reference datasets for treadmill (233) and bicycle ergometer-based (234) CPET for the U.S. population. According to the classification of Takken et al. (230), however, these studies do not fulfill a sufficient number of criteria to be classified as high-quality datasets. One of the problems observed in the FRIEND registry and many other published reference datasets is the non-population-based approach using CPET data sampled in hospitals rather than recruiting a healthy reference population. Even though the participants included in these studies have not been diagnosed with cardiovascular disease, they probably presented limitations regarding physical performance or exercise tolerance. Nevertheless, establishing a large database is a step in the right direction and in the future, an international database that also includes data from Europe, should be pursued. A narrow focus on VO_{2peak} values in the reference studies persists. There is a need for reference values of other CPET variables that have been shown to be predictive of health and disease, such as oxygen uptake efficiency slope (OUES) or VE/VCO_2 slope (135). Ideally, measured CPET variables are interpreted in the context of an individual, accounting for their age and unique health phenotype, and compared to reference values that provide for the most accurate comparison possible (228).

Therefore, aim 4 addresses the need for accurate reference values for maximal and submaximal CPET parameters in a European population.

1.3.3 Use of CPET for risk stratification in Heart Failure

Established Parameters

For four decades, CPET has been used to gain insights into the pathophysiology of patients with HF (235). Several CPET variables have shown the ability to reflect some key pathologic signs of HF such as a reduced cardiac output, elevated neurohormonal markers, or ventilation-perfusion mismatching (236). A large body of research has described the diagnostic and especially prognostic utility of CPET parameters. As a result of this body of research, current scientific statements support and strengthen the utilization of CPET (125). The amount of data generated in each CPET examination is immense and the procedure's complexity seems to hinder a wider application in practice. The majority of scientific studies, however, provides evidence for a relatively small number of key variables in the evaluation of patients with HF. A meta-analysis investigating the prognostic significance of CPET in patients with HF describes that VO_{2peak}, VE/VCO_2 and EOV are highly significant markers and that OUES demonstrates promising results (135). Whether these parameters or other parameters beyond these three fulfil the essential criteria to justify use as

surrogate markers (226, 227), was investigated in the included review in this thesis (aim and publication 1).

Most research studies on CPET parameters and HF patients have been performed in patients with reduced ejection fraction and the amount of research performed in patients with HFpEF is considerably smaller. There has been little success in improving prognosis of patients with HFpEF (237). One reason for this could be the lack of research addressing how to best diagnose and stratify those patients by risk (238). Some initial studies on CPET variables and HFpEF in the last years were able to support the prognostic utility of CPET variables for this patient group as well. Guazzi et al. (239) showed that VO_{2peak} and VE/VCO_2 slope represent disease severity in both HFrEF and HFpEF. Further, the prognostic utility of VO_{2peak} in HFpEF was supported by the FIT-CPX project's results (240).

VO_2-kinetics

A CPET variable that has gained attention in the clinical evaluation of HF patients, particularly in older HF patients, is VO_2-kinetics. Most daily PA and mobility tasks performed by older adults require submaximal, rather than maximal oxygen uptake. Individuals from all age groups undergo countless metabolic transitions to submaximal VO_2 daily (241). It is, therefore, not surprising that the relationship between VO_{2peak} and measures of functional disability in older HF patients is at best moderate (242). In mobility or exercise-impaired older adults, such as HF patients, VO_2-kinetics might be more relevant to objectively evaluate exercise limitations and functional declines compared to maximal aerobic exercise capacity (VO_{2peak}). In mobility-impaired older adults without HF, VO_2-kinetics during the onset of and recovery from submaximal exercise has been shown to be more predictive of functional mobility than VO_{2peak} (243). Further VO_2-kinetics delivers critical information about exercise tolerance and functional mobility in HF patients (244).

VO_2-kinetics represents the rate at which aerobic ATP generation adjusts to changes of exercise intensity (241). VO_2-kinetics is traditionally analyzed at the beginning of a constant rectangular load by a monoexponential function (245-247). According to this function, VO_2 rises steeply immediately after an increase in load and then flattens out progressively until it reaches a constant value (steady state) after approximately 120–150 s. VO_2-kinetics can, therefore, be described by the following function:

$$VO_2(t) = VO_2bas + A\,(1 - e^{-t\tau})$$

where $VO_2(t)$ is the increase in O_2 above the baseline at any time *t*; $VO_2(bas)$ is the baseline VO_2 at resting steady state; A is the amplitude of the VO_2 response (the difference between the VO_2 values at rest and during steady state exercise); and τ is the time constant of the response that corresponds to 63% of the amplitude. It should be noted that the assumption of a monoexponential increase of the oxygen curve is a simplification of the actual physiological

process. Thus, the VO_2-kinetics show two or three independent components depending on the intensity domain.

VO_2-kinetics is dependent on the cardiovascular system's ability to rapidly increase or decrease the perfusion of the working muscles (248, 249), as well as on the ability of the muscles to rapidly change the rate of oxygen utilization (250-252). Therefore, by the determination of VO_2-kinetics, critical information about the regulating capacity of the cardiovascular system and the skeletal muscles can be gained (248, 249, 251). VO_2-kinetics affects the oxygen deficit accumulation at the beginning of exercise and the time course of its restoration at the end of exercise (253). The slower the VO_2 on-kinetics is, the larger the oxygen deficit. It is during this period of exercise that energy requirements are supplemented by anaerobic energy sources, such as high-energy phosphates (e.g., phosphocreatine) and anaerobic glycolysis, which leads to an accumulation of fatigue-inducing metabolites, including lactate, hydrogen ion (H+), and inorganic phosphate (Pi) (251, 254). Thus, a prolonged on-response may contribute to exertional dyspnea and exercise intolerance. Fast VO_2-kinetics is, therefore, besides VO_{2peak}, an important determinant of aerobic performance, exercise capacity, and functional mobility and have been analyzed in numerous studies (244, 255-258).

Patients with HF show significantly slower VO_2-kinetics (i.e. higher time constants) compared to healthy volunteers (244, 251, 255, 256, 259, 260). The slowing of VO_2-kinetics in HF is closely related to impaired ventricular-pulmonary vascular function (248, 249) and/or impaired peripheral oxygen utilization (259). Several studies have investigated whether VO_2-kinetics could be a useful marker for risk stratification in HF (244, 253, 256, 260-265). Some studies reported a prognostic value that is even superior to VO_{2peak} (253, 262, 264), whereas others only found moderate or even no additional value of VO_2-kinetics (244, 256, 261, 265).

Beyond the abovementioned findings, two other factors could help this variable to be of interest for the clinical setting. First, its evaluation seems to be possible by the ramp protocol. Second, its determination does not seem to be dependent on reaching maximal exhaustion. VO_2-kinetics is traditionally measured by performing a constant load test at submaximal intensity (241). CPET using a ramp protocol is, however, the preferred method to perform an exercise test in the clinical setting (125), and more than one exercise test is unlikely to be performed due to the additional time required, costs spent, and motivational aspects of the subject. Further, the submaximal constant load test only delivers one potential valuable marker, whereas a CPET using a ramp protocol provides a clinician with several useful parameters for risk stratification and management of patients with HF (125). Therefore, several studies have investigated VO_2-kinetics by the determination of VO_2 off-kinetics after an incremental CPET (244, 256, 261-263, 265). Another approach applied is that the constant load warm-up, typically performed between 3–5 minutes before the ramp protocol of a CPET, is used to determine VO_2 on-kinetics (249). Although these new approaches to determine VO_2-kinetics are promising, the various protocols applied and calculation methods used in the studies are difficult to compare. VO_2 off-kinetics is significantly slower than VO_2 on-kinetics (266, 267), and the reproducibility differs between calculation

approaches (266, 268). These methodological differences between the studies investigating patients with HF might also explain the controversial results observed. Therefore, the different protocols and calculation approaches determining VO_2-kinetics during a standard ramp protocol should be analyzed and compared according to their potential in the risk stratification of HF patients.

Ichikawa et al. (269) found that when evaluating VO_2 off-kinetics of patients with HF, the level of exhaustion has no influence on the half time (T1/2) of the VO_2 recovery-period as long as the patients reached their individual anaerobic threshold. These findings are of high relevance because the early determination of exercise tests due to a lack of motivation, familiarization, or discomfort to perform maximal exercise can be observed regularly in clinical practice. Whether VO_2-kinetics can be used as a potential substitute when the determination of VO_{2peak} was unsuccessful (no VO_2 plateau observed and secondary exhaustion criteria not fulfilled) requires further investigation and is described in aim 5 of this thesis.

1.3.4 Physical Fitness Components Beyond CRF in the Clinical Setting

The decline of individual physiological functions often ends in a systemic process that contributes to numerous physiological impairments and diseases with aging (270, 271). For example, age- and hypertension-associated vascular dysfunction has been linked to dysfunction in other areas, including cognitive (272-274) and physical and motor functions (275-277). The ability to perform physical tasks is critical for maintaining overall functional capacity (28, 278, 279), and physical fitness parameters are biomarkers of health among older adults, predicting quality of life, disability, and mortality (279-282). Several studies support a link between CVD risk factors, and limitations in physical fitness (283-285). As described in chapter 1.1.3, physical fitness is a set of attributes including cardiovascular endurance, muscle strength/power, and neuromuscular coordination that people have or attempt to achieve in order to carry out daily tasks without undue fatigue (57). The assessment of physical fitness components in clinical practice has focused either on cardiorespiratory fitness, especially in the field of cardiology and pulmonology. On the other hand, geriatric medicine evaluates physical fitness components in the context of sarcopenia, dynapenia, and frailty. Here, the focus is on strength assessments, such as hand grip strength or basic mobility assessments.

The following fitness parameters beyond CRF have been used in research studies or are applied in the context of health assessment or prediction in the clinical setting and seem of interest.

Low muscle strength assessed by an isokinetic dynamometer has been described as a powerful predictor of future loss of mobility and independence in well-functioning older adults (85). Manini et al. (281) defined cutoff points for knee extension strength on an isokinetic dynamometer to identify older adults at high and low risk for future mobility limitation. Force assessments using an isokinetic dynamometer are, however, time and cost intense. Grip strength decrease with advancing age (286) and a low relative grip strength are strongly associated with old-age disability

and life expectancy (49). Both grip and leg strength have been shown to be inversely associated with mortality (287). Not surprisingly, muscle strength seems to be a better predictor than muscle mass (288). Because of the ease of measurement and the strong association between grip strength and health outcomes, this measure has been one of the most popular physical fitness parameters beyond CRF in clinical practice, specifically as an indicator of dynapenia (289-291). Recent research shows, however, that the prediction of mortality and hospitalization based on different upper and lower limb measurements is superior to the measurement at one location (292), whereby the predictive power depends on the disease (293). In addition to handgrip strength, a simple measurement of lower body strength might, therefore, be valuable in clinical assessment. Balogun et al. (294) demonstrated that lower-limb muscle strength, as assessed by a simple dynamometer, appears to be more important than muscle mass for health-related quality of life. The used method in the study to evaluate lower-limb muscle strength can be easily applied in clinical practice.

The assessment of muscle power has been evaluated for research purpose with dynamic contractions, with peak muscle power occurring at approximately 70% of 1 repetition maximum (295, 296). However, there has not been universal agreement in a standardized methodology and it is, thus, difficult to define specific cutoff points for low muscle power (28, 297). Several studies found that leg extensor power correlated with functional performance (298-300). Because it has been described that not only strength decreases in increasing age but also vertical velocity during a countermovement jump, muscle power and jump performance are substantially reduced (296, 301). Muscle power decreases more significantly from the age of 40 years onward, a phenomenon that can only partly be explained by the loss of muscle mass (302). Muscle power is a stronger predictor of developing functional limitations than muscle strength (298, 299). Further increases in muscle power improve functional measures more than increases in muscle strength (303, 304). This finding seems highly relevant for clinical practice. Based on the available studies, it can be hypothesized that an evaluation of muscle power using a countermovement jump might be a useful, yet relatively easily applied measurement.

Neuromuscular coordination is typically assessed by balance testing and gait analysis. In older people, gait speed is a strong independent predictor of mortality (279). In the UK Biobank study, habitual walking speed was found to be one of the strongest predictors of mortality in both sexes (305). In clinical settings, gait speed is typically hand stopped and further gait characteristics are visually assessed. In contrast, in research settings, the gold standard technology involves optoelectronic systems (306) that offer high accuracy. Unfortunately, such systems can only be used in large laboratories because they are expensive and space consuming (307). The change in walking speed in older people is preceded by other changes in gait characteristics such as gait variability, cadence, stride length, and stride width that begin to occur in middle age (308, 309). Therefore, inertial sensors have been introduced lately to measure these parameters not only in research settings. Inertial sensors are relatively

inexpensive and simple to handle, and they can assess speed, spatiotemporal parameters, and foot kinematics of walking for real-time gait analysis in clinical practice (310). Gait parameters beyond walking speed may represent highly suitable indicators of physical fitness and functional status in advanced age in health or disease and are now relatively easily assessed by these inertial sensors (309, 311, 312). Balance and the control of posture are typically assessed as the time during which the subjects are able to remain standing in a specific foot position such as side-by-side, semi-tandem and tandem position. To increase the objectivity and reliability of the postural control measure, the stands can be performed on a force plate, and the sway path of the center of pressure can be determined (313). Even though not assessed as frequently as a health marker, postural balance presents one of the domains of physical fitness and functional capacity. Among older adults, poor balance is associated with difficulties in activities of daily living (314), and it may predict placement in residential care (315) and even poor survival (316, 317). It could be one of several variables to assess in order to gather comprehensive information of an individual's physical fitness.

Assessing the various aspects of physical fitness across the lifespan is a complex measurement task. As described above, several parameters seem crucial, and approaches towards a comprehensive assessment should be made. An early and widely recognized approach is the short physical performance battery (SPPB) based on three tests: walking speed, repeated chair stand, and a balance test (318, 319). The test seems to miss several critical components of physical fitness; foremost, cardiovascular endurance. Further, the test was developed to assess individuals over 70 years of age (319). It is, therefore, unlikely that the test battery provides essential information on physical fitness in middle-aged adults. A more recent initiative for the assessment of physical fitness/motor function in health settings is the National Institutes of Health (NIH) Toolbox and its motor assessment part (320). The NIH toolbox assesses the following subdomains of fitness: dexterity, strength, balance, locomotion, and endurance and provides, therefore, a more complete test. Similar to the SPPB, the NIH Toolbox includes several tests that do not seem to be suitable for young and middle-age individuals, such as the 4-meter walk to test locomotion or the 2- and 6-min walk test as aerobic endurance measures. Further improvement of the tests, such as different versions depending on the measurement methods available or the amount of underlying physiological information required, could lead to a wider application of the instrument beyond the U.S.. Recently, McKay et al. provided an important contribution to the field of physical fitness testing and reported reference values of 12 functional outcome measures across the lifespan (321).

With the increasing number of individuals aged over 65 years and particularly the high number of patients in this age group suffering from multimorbidity, the assessment of a combined approach of physical fitness components is expected to gain further attention. Research studies have primarily focused on the assessments of a single variable or one domain of physical fitness. To the author's knowledge, there are only a very small number of cohort studies that have assessed

several components of physical fitness and their association with health outcomes (322, 323). Because many individual physical fitness parameters have been shown to have predictive values for health outcomes in healthy people but also individuals with chronic diseases, a combined approach seems highly promising. Therefore, the COmPLETE Project attempts to address this research gap (aim and publication 2). Further, in publication 6, novel statistical approaches shall be applied to combine several physical fitness markers and compare the composite measure between health and HF.

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Chapter 2

Aims and Hypotheses

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The aims and hypotheses of this Ph.D. project were:

Aim 1: To perform a narrative review to test the hypothesis that ventilatory gas exchange variables of CPET are useful for risk stratification and management of HFrEF

Aim 2: To design a study that can identify physical fitness and cardiovascular biomarkers that best resemble underlying cardiovascular risk with age and to examine which physical fitness markers are impaired with progressing age and in heart failure.

Aim 3: To test the hypothesis that high and age-dependent secondary exhaustion criteria are required to balance type I and type II errors in the determination of VO_{2peak}.

Aim 4: To provide CPET reference values for maximal and submaximal parameters across the adult age spectrum of a healthy European cohort. Further, to test the hypotheses that (1) there is a positive correlation between health- and performance-related CPET parameters and moderate and vigorous PA, and (2) reference values for VO_{2peak} from this healthy European cohort are higher compared to other previously published reference studies.

Aim 5: To test the hypotheses that (1) heart failure patients demonstrate slower VO_2 on- and VO_2 off-kinetics compared to healthy participants, (2) differences between calculation approaches of VO_2-kinetics are prevalent, and (3) VO_2-kinetics can provide additional value beyond that of VO_{2peak}.

Aim 6: To test the hypothesis that a significant health distance of physical fitness components (i.e., cardiovascular endurance, muscular strength, and neuromuscular coordination) can be observed between patients with heart failure and healthy individuals

Chapter 3

Publication 1

The Role of Gas Exchange Variables in Cardiopulmonary Exercise Testing for Risk Stratification and Management of Heart Failure with Reduced Ejection Fraction.

Authors:

Jonathan Wagner[1], Piergiuseppe Agostoni[2], Ross Arena[3], Romualdo Belardinelli[4], Daniel Dumitrescu[5], Alfred Hager[6], Jonathan Myers[7], Rainer Rauramaa[8], Marshall Riley[9], Tim Takken[10], Arno Schmidt-Trucksäss[1]

[1] Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland
[2] Centro Cardiologico Monzino, IRCCS, Milano, Italy & Department of Clinical sciences and Community health, Cardiovascular Section, University of Milano, Milano, Italy
[3] Department of Physical Therapy, University of Illinois at Chicago, Chicago, IL, USA
[4] Department of Cardiovascular Sciences, Cardiac Rehabilitation Lancisi, Ancona, Italy
[5] Herzzentrum der Universitaet zu Koeln, Cologne, Germany
[6] Department of Pediatric Cardiology and Congenital Heart Disease, Technical University of Munich, Germany
[7] Cardiology Division, VA Palo Alto Health Care System and Stanford University, Palo Alto, CA, USA
[8] Foundation for Research in Health, Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland
[9] Department of Medicine, Royal Victoria Hospital, Belfast, Northern Ireland
[10] Department of Pediatrics, University Medical Center Utrecht, Utrecht, Netherlands

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Chapter 4

Publication 2
Functional Aging in Health and Heart Failure: the COmPLETE Study

Authors:

Jonathan Wagner[1], Raphael Knaier[1], Denis Infanger[1], Konstantin Arbeev[2], Matthias Briel[3,4], Thomas Dieterle[5], Henner Hanssen[1], Oliver Faude[1], Ralf Roth[1], Timo Hinrichs[1], Arno Schmidt-Trucksäss[1]

[1] Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland
[2] Biodemography of Aging Research Unit (BARU), Social Science Research Institute, Duke University, Durham, NC, USA
[3] Department of Clinical Research, Basel Institute for Clinical Epidemiology and Biostatistics, University Hospital Basel, Basel, Switzerland
[4] Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, ON, Canada
[5] University Clinic of Medicine, Cantonal Hospital Baselland, University of Basel, Liestal, Switzerland

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STUDY PROTOCOL Open Access

Functional aging in health and heart failure: the COmPLETE Study

Jonathan Wagner[1], Raphael Knaier[1], Denis Infanger[1], Konstantin Arbeev[2], Matthias Briel[3,4], Thomas Dieterle[5], Henner Hanssen[1], Oliver Faude[1], Ralf Roth[1], Timo Hinrichs[1] and Arno Schmidt-Trucksäss[1*]

Abstract

Background: Cardiovascular (CV) diseases including heart failure are the leading causes of morbidity, with age being the primary risk factor. The combination of age-related organic functional impairment and reduced physical fitness can drastically impact an individual's healthspan. One's lifespan can potentially be prolonged by the preservation or improvement of physical fitness. However, it remains unclear as to which biomarkers are most suitable for distinguishing between healthy aging and the impaired organ function associated with heart failure. Therefore, a comprehensive assessment of the components of physical fitness and CV function will be performed to identify the most important factors contributing to aging in relation to both health and disease.

Methods: This cross-sectional investigation will consist of two parts: COmPLETE-Health (C-Health) and COmPLETE-Heart (C-Heart). C-Health will examine the aging trajectories of physical fitness components and CV properties in a healthy population sample aged between 20 and 100 years ($n = 490$). Separately, C-Heart will assess the same markers in patients at different stages of chronic heart failure ($n = 80$). The primary outcome to determine the difference between C-Health and C-Heart will be cardiorespiratory fitness as measured by cardiopulmonary exercise testing on a bicycle ergometer. Secondary outcomes will include walking speed, balance, isometric strength, peak power, and handgrip strength. Physical activity as a behavioural component will be assessed objectively via accelerometry. Further, CV assessments will include pulse wave velocity; retinal, arterial, and venous diameters; brachial and retinal arterial endothelial function; carotid intima-media thickness; and systolic and diastolic function. The health distances for C-Health and C-Heart will be calculated using the methodology based on statistical (Mahalanobis) distance applied to measurements of quantitative biomarkers.

Discussion: This research seeks to identify physical fitness and CV biomarkers that best resemble underlying CV risk with age. Further, it will examine which physical fitness markers are impaired most in heart failure. The presented integrative approach could define new recommendations for diagnostic guidance in aging. Ultimately, this study is expected to offer a better understanding of which functional characteristics should be specifically targeted in primary and secondary prevention to achieve an optimal healthspan.

Keywords: Aging, Fitness, Exercise, Vascular function, Heart failure

Background

The population of industrialized countries is aging. By the year 2050, at least one-quarter of the population in developed countries will be older than 65 years of age [1]. Similarly, life expectancy is projected to increase in industrialized countries with probability rates of at least 65% for women and 85% for men, respectively [2]. A more significant part of the projected gains in life expectancy at birth will be due to enhanced longevity above the ages of 50 and 65 years [2]. In recent decades, aging and associated chronic diseases have become an increasing concern for our society and the health care system [3]. The evolutionary 'unforeseen' gain in lifespan would not be a catastrophe if it were a gain of healthy years of life. However, since the leading causes of early mortality, such as death around birth and death from infections, can largely be prevented by modern medicine, the presence of chronic noncommunicable diseases at

* Correspondence: arno.schmidt-trucksaess@unibas.ch
[1]Department of Sport, Exercise and Health, University of Basel, Birsstrasse 320 B, 4052 Basel, Switzerland
Full list of author information is available at the end of the article

middle age to advanced age progressively gain in importance. Nonetheless, cardiovascular (CV) disease, which includes heart failure, is the leading cause of morbidity, and chronological aging is its primary risk factor [4, 5]. The prevalence of chronic heart failure (CHF) in the developed world has increased significantly in the last three decades [5]. CHF is associated with high morbidity and mortality, making this chronic condition a significant health care concern [5].

A concept to counteract the traditional way of thinking of age-associated chronic diseases was introduced as early as 1980 [6]. The goal of this concept was to increase the years free of disease or to shorten the disease phase during the final part of one's life. The compression of morbidity is to be attained by optimal prevention [6] and presumes that illness is absent up until a certain point in life and then is present for a brief period thereafter until the end of one's life. Since this concept neither adequately takes into account the aging of the organism nor considers the functional limitations that may present before the onset of a disease, the term 'healthspan' was recently introduced [7–9]. One's healthspan is defined as a period of relatively healthy aging followed by a period of age-related diseases and disabilities [7–9] (Fig. 1 a). During the period of relatively healthy aging (orange to red area in Fig. 1 b), the functions of various organ systems are already somewhat restricted, including the CV system. Vice versa, the diminishing function of the organ systems due to age increases the risk of chronic diseases.

Curative medicine prolongs a lifespan but may also be associated with a longer time of disability (Fig. 1 b). An optimal healthspan may be achieved through the combination of curative medicine as well as an improvement of one's physical fitness components (Fig. 1 c).

Physical fitness is defined as a set of attributes (e.g., endurance capacity, muscle strength, neuromuscular coordination) that people have or attempt to achieve in order to carry out daily tasks without undue fatigue [10, 11]. Reduced physical fitness is accompanied by a reduction in the use of the organ systems involved in physical activity and appears to be equally important as the aging of the organs themselves in the concept of healthspan. The deterioration of the main components of physical fitness (i.e., endurance capacity, muscle strength, and neuromuscular coordination) due to inactivity or insufficient physical activity is associated with a lower capacity of the CV system, skeletal muscles, and the neuromuscular system [12, 13]. The final state of this unfavourable process is generally regarded as a frailty or disability status [14].

The age-associated functional limitations of the organ systems and the lower level of physical fitness due to reduced physical stimuli cannot clearly be separated from one another but rather interact with one another in the aging process [15]. In addition, acute or chronic illnesses affect physical fitness and can accelerate the effects of reduced use and thus cause the organism to reach the threshold of frailty more quickly [7, 16]. On the other hand, an improvement of the physical fitness can alleviate the severity of occurring diseases. The overarching idea related to these two processes (decline of physical fitness and age-associated functional limitations of the organ systems) is now to use their interaction to counteract the development of chronic diseases by maintaining or improving physical fitness. This is all the more likely because physical activity and training on numerous paths can influence the development of the disease and its course, which has been proven many times [17, 18].

The combination of age-related organic functional impairment and reduced physical fitness can debilitate the healthspan of an individual. Separately, the healthspan can potentially be prolonged by the preservation or improvement of physical fitness with advancing age.

The rationale for a combined assessment of the aforementioned components of physical fitness arise from

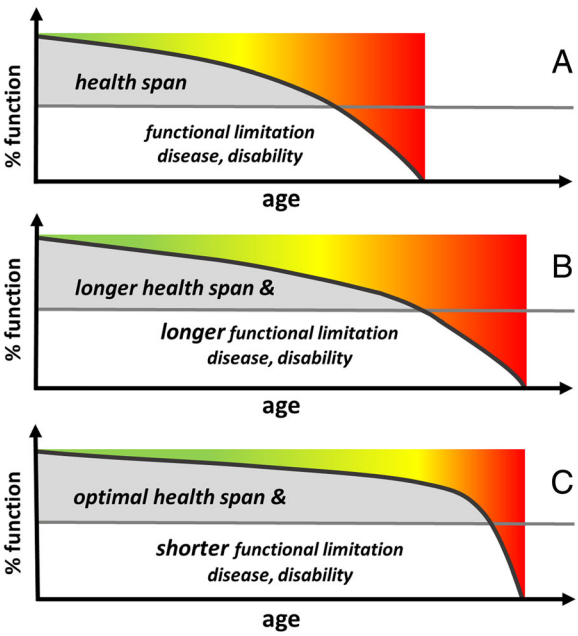

Fig. 1 Healthspan. Healthspan is the period of life without functional limitations, chronic diseases. and disability but with a beginning loss of functional capacity symbolized by a change from green to orange colour (**a**). This period may be prolonged by curative medicine (**b**), but longer disability may also occur. Optimal healthspan can potentially be achieved by preserving or improving the functional abilities of the organism and therefore lead to a shorter period of functional limitations, chronic diseases, and disability at the end of life (**c**). Good physical fitness is thought to contribute essentially to optimal healthspan. Adapted from Seals et al. [7]

their individual and separate predictive values for all-cause mortality and CV mortality [19–22].

The isolated assessment of physical function would be, however, worthless to perform in the described cross-sectional study without robust surrogate health markers. As it is impossible to test all physiological organ functions against the physical function parameters, this study instead focuses on vascular health and CV disease surrogate markers to characterize organ function. CV biomarkers have been shown to be excellent markers for overall physiological status during aging. Arterial dysfunction increases the risk of several common chronic disorders with advanced age such as coronary disease, kidneys disease, stroke, cognitive impairment, Alzheimer's disease, motor disorder, and heart failure [23]. Impaired physical fitness is a 'gateway' to early vascular aging, vascular dysfunction, and an increased risk for CV disease [19].

It is still unclear as to which CV and physical fitness biomarkers distinguish best between healthy functional and dysfunctional aging. Also, the potential variations with age of these markers in healthy people and in people with early stages of chronic disease such as heart failure are largely unknown (Fig. 2). A thorough assessment of vascular health, CV diseases surrogate markers, and fitness parameters is needed to understand the relationships and interplay between physiological function and physical fitness.

Physical fitness components and aging in health and heart failure

Endurance capacity

Several prospective studies on endurance capacity show a strong predictive value for overall and CV mortality. Kodama et al.'s meta-analysis [19] involving 102,980 participants and 6,910 outcomes for all-cause mortality revealed a substantial contribution of cardiorespiratory fitness that was independent of classical risk factors. As compared with participants with high fitness, those with low fitness had a 70% higher risk for all-cause mortality and a 56% higher risk for CV mortality. A recently published follow-up study of 22,878 participants with a baseline mean [standard deviation (SD)] age of 47.4 (10.3) years and a follow-up of 9.2 (SD: 4.1) years and 505 deaths added cardiorespiratory fitness to the Systematic Coronary Risk Evaluation (SCORE) risk model. The study revealed a substantial improvement of the combined risk model of SCORE. Cardiorespiratory fitness alone was also a better risk predictor than the SCORE value alone [20]. People with high SCORE values and low fitness (metabolic equivalents < 11) showed a relative hazard ratio for death of 35.6 versus the low SCORE value and high fitness group. However, those with high SCORE values but high fitness (metabolic equivalents ≥11) had only a hazard ratio of 8.5. A recent analysis of a Finnish population confirmed these results for incident fatal myocardial infarction and CHF. From 2,089 participants with a baseline age of 53.1 (SD: 4.9) years and a follow-up of 19.1 (SD: 8.4) years, the rate of fatal myocardial infarction ($n = 522$) and nonfatal heart failure increased with a reduction in cardiorespiratory fitness [24]. The Mayo Clinic's patient database recently included cardiorespiratory fitness in a treadmill score in the risk prediction for CV mortality. Out of 58,020 participants with a mean age of 53 years (49% women), 6,456 patients (11%) died by the median follow-up point over 10 years. When cardiorespiratory fitness was considered in the risk prediction, traditional CV risk

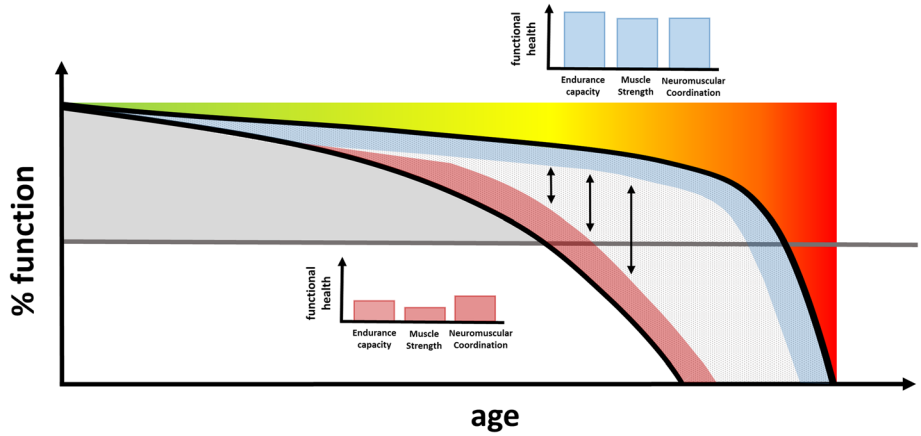

Fig. 2 Health distance. Graphical illustration of the health distance. Healthy individuals are potentially located at the upper limit of the healthspan (blue area). Individuals with heart failure are potentially located at the lower limit of the healthspan (red area) and reaching the line of functional limitation (grey line) earlier as compared with healthy individuals. Double arrows denote the health distance between healthy individuals and patients with chronic heart failure. The upper bar chart represents the better components of physical fitness of healthy individuals, while the lower bar chart represents the worse components of physical fitness of a patient with chronic heart failure. Modified from Seals et al. [7]

factors did not contribute incrementally to survival discrimination [25].

In heart failure, the most important and often used parameter for risk prediction is peak oxygen uptake ($\dot{V}O_2$peak). Different studies have shown an incremental value of $\dot{V}O_2$peak to other risk factors [21, 26, 27]. $\dot{V}O_2$peak and percent-predicted $\dot{V}O_2$peak added prognostic value when included in a multivariate Cox regression analysis with standard risk variables including New York Heart Association (NYHA) functional class and left ventricular ejection fraction (EF) (LVEF) [21]. This incremental value of $\dot{V}O_2$peak has also been shown for heart failure with a preserved EF (HFpEF) [28]. The relationship of the ventilation to carbon dioxide production slope ($\dot{V}E/\dot{V}CO_2$ slope) is a second independent predictor for CHF hospital admission after consideration of recognized clinical variables such as age, NYHA functional class, EF, body mass index, creatinine, and B-type natriuretic peptide (BNP) [27]. The variables of cardiopulmonary exercise testing (CPET), exercise oscillatory ventilation (EOV), oxygen uptake efficiency slope (OUES), and the partial pressure of end-tidal CO_2 ($PETCO_2$) constitute additional variables that have all been prospectively validated [29]. As described in a recent review [29], each variable reflects in part a different pathophysiologic feature of heart failure with reduced EF (HFrEF) and therefore recommends the parallel assessments of these five CPET variables in those patients.

Muscle strength

Muscle strength is determined by muscle mass and fibre composition as well as intra- and intermuscular coordination. Muscle power decreases more significantly in men and women from the age of 40 years onward, a phenomenon that can only partly be explained by the loss of muscle mass [30]. Rather, the change in fibre composition is decisive for the contraction velocity that determines muscle power. Muscle strength reaches its peak around the age of 30 years and remains almost constant until the age of 50 years and then decreases continuously between 2 and 5% per year depending on the individual's age [31]. The loss of muscle mass and strength that occurs with advanced age is defined as sarcopenia. Traditionally, sarcopenia has been defined as a loss of appendicular muscle mass of less than two SDs below the mean muscle mass of a person aged 35 years old [32]. Other thresholds used for the definition include low grip strength or a low usual gait speed of less than 0.8 to 1 m/s [14, 33, 34]. Sarcopenia syndrome is characterized by a progressive and generalized loss of skeletal muscle mass and strength and associated with an increased risk of adverse outcomes such as physical disability, poor quality of life, and death [35]. Grip as well as leg strength have been shown to be inversely associated with mortality [36]. Muscle strength seems to be a better predictor than muscle mass [22]. Recent research shows that the prediction of mortality and hospitalization based on different upper and lower limb measurements is superior to the measurement at one location [37], whereby the predictive power depends on the disease [38].

Skeletal muscle mass is reduced in heart failure with reduced EF by approximately 20% and in heart failure with preserved EF by approximately 10%, respectively [35, 39]. According to the NYHA classification scheme, significant differences in muscle strength have also been demonstrated in patients with heart failure, with severity levels differing. For example, the strength of the leg extensors and flexors as well as the hand grip strength are significantly decreased in patients with NYHA functional class III versus those with NYHA functional class I [40].

Furthermore, a significantly lower strength of the *M. quadriceps* femoris and of the hand grip is found in heart failure patients both with and without muscle wasting [39, 41]. Even in the mild stages of heart failure, leg strength is already limited [41]. In addition to the reduction of muscle mass, a reduction of type I fibres can be observed in the skeletal muscles [42]. This applies to patients with either HFrEF or HFpEF as compared with healthy controls [43]. These changes in the peripheral musculature seem to be mainly responsible for the reduction of endurance capacity. An improvement in $\dot{V}O_2$peak is found to be 27% by the muscle oxygen diffusion capacity and only about 7% by the cardiac output [44].

In summary, muscle wasting and sarcopenia in people with heart failure may be noninvasively detected through impaired physical fitness such as reduced muscle strength.

Neuromuscular coordination

Neuromuscular coordination is typically assessed by balance testing and gait analysis [45]. In older people, gait speed is a strong independent predictor of mortality [46]; the United Kingdom Biobank study confirmed that habituary walking speed is one of the strongest predictors of mortality in both sexes [47]. However, the change in walking speed in older people is preceded by other changes in gait characteristics such as gait variability, cadence, stride length, and stride width that begin to occur in middle age [48, 49]. The relationship between muscle strength and gait characteristics were investigated in the Baltimore Longitudinal Study of Aging in middle-aged (32–57 years), old-age (58–78 years), and 'oldest'-age (79–93 years) participants. A reduction in walking speed between middle age and old age was found to be a result of a reduction in muscle strength and neuromuscular coordination [13]. Beyond the age of 60 years, slow walking speed is an independent and strong predictor of poor

health status. Further, poor balance and mobility are significant predictors of mortality in predisabled women aged 75 years and older [50].

In heart failure patients, gait speed is an independent predictor of mortality [51]. In a recent study, gait speed was associated with a lower risk for all-cause mortality independent of age; EF of less than 20%; and other parameters such as systolic blood pressure, anaemia, and the absence of beta-blocker therapy [52].

Cardiovascular phenotype in health and heart failure

Vascular biomarkers

The assessment of vascular biomarkers is essential in order to research the potential impact and link between physical fitness and physical activity on vascular function and/or vascular impairment. In addition to a broad spectrum of measures of physical functioning, the assessment of vascular biomarkers and quantification of the burden of atherosclerosis in asymptomatic, apparently healthy individuals and heart failure is also of considerable importance.

In 60% of asymptomatic individuals aged 68.9 ± 6.0 years, subclinical atherosclerosis was detectable in two vascular beds (carotid and coronary arteries) [53]. Additionally, the MONICA–Augsburg study found at least one plaque in the carotid or femoral artery in 51.8% of men and 36.3% of women assumed to be healthy [54]. Since few studies have shown an inverse association of prevalent subclinical vascular disease and components of physical fitness [55–58], it is crucial to assess the atherosclerotic burden in parallel.

Arterial stiffness

Arterial stiffness increases with age and is measured as pulse wave velocity (PWV). The increase in arterial stiffness accelerates in particular beyond the age of 60 years [59]. The increase in PWV causes an increase in central arterial blood pressure and is thought to cause left ventricular hypertrophy and impaired left ventricular diastolic function [60]. Increased PWV was identified as an independent predictor of CV and all-cause mortality in a prior meta-analysis of individual data [61]. Vigorous physical activity and higher cardiorespiratory fitness are inversely associated with age-related arterial stiffening [55]. Improvements in cardiorespiratory fitness may, therefore, be a useful measure for preventing age-related increases in arterial stiffness [62].

In HFpEF, brachial–ankle PWV (baPWV) demonstrated a J-shaped association with CV events in a Japanese study. The authors suggested a low baPWV in HFpEF patients reflected aggravated cardiac diastolic dysfunction, while a high baPWV indicated large-artery stiffening [63]. A recent prospective study performed among heart failure patients from the Health, Aging, and Body Composition study did not show any independent predictive value of central PWV for risk prediction [64]. However, it is still possible that other parameters of central haemodynamics—for example, central pulse pressure—may have a predictive value. Another study showed an improvement in physical function in heart failure patients following vasodilator therapy and improvements in central haemodynamics [65]. PWV is predictive of left ventricular hypertrophy and CV events in hypertensive patients regardless of hypertension status (controlled or uncontrolled blood pressure) [66]. Therefore, PWV represents a promising marker with which to characterize and distinguish healthy aging and early stages of heart failure.

Brachial artery flow-mediated dilatation

Impaired flow-mediated dilation (FMD) reflects endothelial dysfunction as an early marker of atherosclerotic arterial damage [59]. In addition to being sensitive to changes in lifestyle [67], FMD is associated with traditional risk factors, CV diseases, and heart failure and predicts both CV events and all-cause mortality [59]. Impaired FMD is associated with reduced exercise capacity, aging [62, 68], and heart failure. Impaired FMD in heart failure improves with drug therapy [69].

Retinal vessels

Retinal vessels are regulators of the local cerebrovascular blood flow, are valid and robust microvascular surrogate biomarkers of CV risk and mortality, and can be analysed very effectively [70, 71]. Large cohort studies have previously shown that narrower retinal arterioles, wider retinal venules, and a resulting lower arteriolar-to-venular diameter ratio (AVR) are associated with an increased risk and severity of hypertension [72, 73], risk of stroke [71, 74] and CV morbidity and mortality in older participants [75]. In older adults, obesity is associated with retinal venular widening, and a lower AVR can be explained by the association of low-grade inflammation with obesity [76, 77].

To date, very little is known about the association of physical fitness with microvascular health. The only available exercise intervention study showed that higher physical fitness levels are associated with higher retinal AVR and that exercise-induced arteriolar dilatation as well as venular constriction lead to a significantly improved AVR in middle-aged lean and obese individuals [78]. In particular, the obese group seemed to benefit the most from the exercise training, with significantly dilated retinal arteries observed after a 10-week exercise program [78]. Dynamic retinal vessel analysis is a new innovative diagnostic method for the assessment of cerebrovascular endothelial function. It has recently been shown that retinal endothelial function, assessed as

retinal vessel dilatation in response to flicker light, is impaired in prediabetic and diabetic patients as well as individuals with CV risk and CHF [79, 80].

To conclude, macro- and microvascular arterial properties are predictive of CV events and may help to discriminate between healthy individuals and patients with heart failure. Among motor functional components, endurance capacity specifically is inversely associated with vascular properties.

Methods/design

Objectives

This project seeks to do the following:

Specific Aim 1

Determine the trajectories of physical fitness components of healthy aging by the measurement of endurance capacity, muscular strength, and neuromuscular coordination in a healthy population sample aged between 20 and 100 years.

Specific Aim 2

Determine the health distances of physical fitness components (i.e., endurance capacity, muscular strength, and neuromuscular coordination) for patients with heart failure and age-matched healthy men and women.

Study design

The COmPLETE study is a cross-sectional study and consists of two parts, COmPLETE-Health (C-Health) and COmPLETE-Heart (C-Heart).

Hypotheses

Hypothesis 1 (C-Health): There is an apparent effect of age on all physical fitness components (endurance capacity, muscular strength, and neuromuscular coordination) in persons aged 20 to 100 years old.

Hypothesis 2 (C-Heart and C-Health): The health distances of components of physical fitness (endurance capacity, muscular strength, and neuromuscular coordination) for patients with heart failure and healthy individuals are not equal (Fig. 2).

Recruitment

In C-Health, recruitment will be performed until a total number of 490 participants with a valid cardiopulmonary exercise test (CPET) as our primary outcome are included. All participants will be recruited in the area of Basel, including 35 males and 35 females per age category (i.e., 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80+ years of age). The recruitment will be based on unaddressed letters sent to randomly chosen districts of the 11 neighbourhoods of the city of Basel and 15 municipalities of the district of Arlesheim. Arlesheim is one of the five districts of the canton of Basel-Country. These districts represent both rural and urban environmental conditions. Potential participants in the age category of 80+ years will likely be underrepresented and only make up approximately 6% of the population living in this area only. Therefore, the recruitment will be widened for this group. After finishing the recruitment of all other age categories, recruitment letters will be sent to specifically targeted neighbourhoods with a higher percentage of older inhabitants but still involving rural and urban districts. Further measures for recruiting participants aged 80+ years might be discussed if necessary. A telephone questionnaire screening of potential participants before making appointments will be conducted. Final eligibility will be confirmed onsite on the day of examination.

C-Heart will include 80 heart failure patients characterized according to criteria named below. C-Heart participants will be recruited using various recruiting strategies such as recruiting letters, flyers; cooperation with a local cardiology unit of a hospital; and cooperation with internists and cardiologist in the area of Basel, Switzerland. The cardiologists and internists will provide potential participants with an information sheet about the goal and the conduct of the present study and identify interested patients for eligibility based on the inclusion and exclusion criteria. Potential participants through other recruitment channels will be reviewed for eligibility per telephone interview. For all potential C-Heart participants, the final eligibility will be confirmed onsite on the day of examination by a physician.

Inclusion criteria

C-Health:

- Healthy men and women aged 20–100 years
  - Body mass index < 30 kg/m^2
  - Nonsmoker

C-Heart:

- Stable CHF (treated patient with symptoms and signs that have remained generally unchanged for at least one month) characterized according to the European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure [81], as follows:
  1. HFrEF (LVEF < 40%)
  2. HFmrHF (LVEF 40–49%) and NT-proBNP > 125 pg/mL and relevant structural heart disease or diastolic dysfunction

3. HFpEF (LVEF ≥50%) and NT-proBNP > 125 pg/mL and relevant structural heart disease or diastolic dysfunction

Exclusion criteria

C-Health:

- Age younger than 20 years; manifest exercise limiting chronic disease (e.g., myocardial infarction; stroke; heart failure; lower-extremity artery disease; cancer with general symptoms; diabetes; clinically apparent renal failure; severe liver disease; chronic bronchitis GOLD stages II to IV; osteoporosis), women with known pregnancy or breastfeeding; drug or alcohol abuse; hypertonic blood pressure of more than 160/100 mmHg; compromising orthopaedic problems; Alzheimer's disease or any other form of dementia; inability to follow the procedures of the study (e.g., due to language problems, psychological disorders, dementia of the participant); diseases regarded as an absolute contraindication for maximal exertion; and current or past smoking status.

C-Heart:

- Age younger than 20 years; women with known pregnancy or breastfeeding; drug or alcohol abuse; inability to follow the study procedures (e.g., due to language problems, psychological disorders, etc.); unstable angina pectoris; uncontrolled brady- or tachyarrythmia; permanent atrial fibrillation; severe uncorrected valvular disease; acute myocardial infarction or coronary syndrome; transient ischemic attack or stroke occurring less than three months prior; clinically significant concomitant disease states (e.g. uncontrolled hypertonic blood pressure); clinical evidence of current malignancy with exception of basal cell or squamous cell carcinoma of the skin and/or cervical intraepithelial neoplasia; currently receiving systemic chemotherapy and/or radiotherapy; significant musculoskeletal disease other than that associated with heart failure limiting exercise tolerance; active infection; immunosuppressive medical therapy; life-expectancy of less than six months; and prevalence of a disease regarded as an absolute contraindication for maximal exertion.

Setting

The study will be carried out at the Department of Sport, Exercise, and Health at the University of Basel, Switzerland. This study is funded by the Swiss National Science Foundation (grant no. 182815) and was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017–01451).

Study procedures and ethical considerations

The research project will be carried out in accordance to the research plan and with principles enunciated in the current version of the Declaration of Helsinki and the guidelines of Good Clinical Practice (World Medical Association, 2013).

The Ethics Committee of Northwestern and Central Switzerland and regulatory authorities will receive annual safety and interim reports and will be informed about study stop/end in agreement with local requirements. This study protocol was designed according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. All measurements and procedures applied in this study are noninvasive.

All participants will be briefed verbally and will receive information approved by the local ethics committee giving details on the study procedures. All participants will have to sign a consent form and will be informed about their right to withdraw from the study without any consequences. Retinal vessel analysis will include mydriasis of one eye. Using a mydriaticum (tropicamide 0.5%), the pupils will be dilated to enable retinal vessel analysis. The eye drops can cause temporary discomfort, often a burning sensation for one to two minutes. Flicker light exposure can potentially cause slight headaches.

Methods and course of measurements

The measurements will be carried out within approximately 3.5 to 4 h for visit 1 and 60 min for visit 2. The assessment of physical activity will be monitored for 14 days by a wrist-worn accelerometer after visit 1. Retinal vascular assessment will be carried out on a different day (visit 2) that takes 60 min and which is not more than one months apart from visit 1. The precise sequence is shown in Table 1. Standardized procedures will be used to perform all measurements, and the assessment staff will use standardized instructions for all measurements to ensure equal testing conditions for all participants.

Physical fitness components

Endurance capacity: cardiopulmonary exercise testing

An exercise test until maximal exertion using an electromagnetically braked cycle ergometer (Ergoselect 200; Ergoline, Bitz, Germany) will be performed according to one of the following five protocols: a three-minute warm-up will be performed either unloaded with a load of 10 or 20 W for protocols 1 to 3 or with a load of 50 W for protocols 4 and 5; a warm-up will be followed by ramp protocol 1, 2, 3, 4, or 5 with a linear workload increase of 7, 10, 15, 20, or 30 W/min, respectively. The

Table 1 Outcomes assessed in the COmPLETE Study (C-Health & C-Heart)

	Outcome measure	Data Collection Instrument
Before Visit 1		
Telephone interview	General health and chronic disease, part 1[a]	21 items
	Smoking status [a]	3 items
	Physical activity readiness [a]	7 items, Physical Activity Readiness Questionnaire (PAR-Q)
Visit 1		
Questionnaires	Chronotype (1)	14 items, Munich Chronotype Questionnaire (MCTQ) [82]
	Quality of life (2)	8 items, Health related Quality of Life, short form (SF-8) [83]
	Socio-economic status (3)	1 item
	Subjective physical activity (4)	10 items, European Health Interview Survey-Physical Activity Questionnaire (EHIS-PAQ) [84]
		6 items, Global Physical Activity (GPAQ) [85]
	Residential area (5)	3 items
	Use of transportation (6)	3 items
	Life-space (7)	9 items, modified UAB Study of Aging Life-Space Assessment [86]
	Fall history (8)	2 items
	Alcohol consumption (9)	3 items
	Stress (10)	4 items, Perceived Stress Scale (PSS) [87]
	Insomnia (11)	7 items, Insomnia Severity Index (ISI) [88]
	Menstruation cycle (12)	7 items
	General health and chronic disease, part 2 (19)	13 items
	Medication (20)	10 items
Anthropometry	BMI [a] (13)	Weight and height
	WHR (14)	Waist circumference/hip circumference
	Body composition (15)	Four-segment bioelectrical impedance analysis
Macrovascular-Health	Arterial stiffness (baPWV/CAVI) & blood pressure [a] (16)	Noninvasive vascular screening system
	Brachial endothelial function (17)	FMD by ultrasound
	Carotid-intima-media thickness (23)	2D ultrasound instrument
Cardiac Imaging	Systolic and diastolic structure and function (22)	2D echocardiography
Inflammation & Circulating CV Risk Factors	Cholesterol (TC, LDL, HDL), triglycerides (TGA), HbA1c, NT-pro BNP & etc. (21)	Venous blood samples
Physical Fitness Components	Gait (18)	Inertial sensor system
	Power of leg muscles (24)	Countermovement jump on a force plate
	Standing balance (25)	Tandem stance on a force plate
	Handgrip strength (26)	Handheld dynamometer
	Isometric leg strength (27)	Dynamometer
	Cardiorespiratory fitness (28)	Cardiopulmonary exercise testing with breath-by-breath gas analysis
Starting the day after visit 1 for 14 days		
Physical Activity	Objective physical activity	Wrist-worn triaxial accelerometer
Visit 2		
Microvascular-Health	Retinal arterial and venous diameters	Static retinal vessel analysis
	Retinal endothelial function	Dynamic retinal vessel analysis

[a] Used for a check of inclusion criteria. Numbers in brackets indicate the precise sequence of data collection of visit 1
Abbreviations: *BMI* body mass index, *WHR* waist-to-hip ratio, *baPWV* brachial–ankle pulse wave velocity, *CAVI* cardio–ankle vascular index, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *FMD* flow-mediated dilation

three-minute recovery phase will be performed at the same wattage as the warm-up. The protocol will be chosen to achieve a ramp duration of between six and 18 min [89, 90]. Pedalling cadence will be chosen by participants but is required to be more than 60 rpm.

Gas exchange and ventilatory variables will be analysed breath-by-breath continuously using a computer-based system (MetaMax 3B; Cortex Biophysik GmbH, Leipzig, Germany). Every test is preceded by a resting period of three minutes to reach steady-state conditions. The steady-state status will be analysed for the plausibility of $\dot{V}O_2$ (mL/min), $\dot{V}CO_2$ (mL/min), $\dot{V}E$ (L/min), ventilatory equivalents for oxygen and carbon dioxide ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$, respectively), and end-tidal gas tensions for oxygen and carbon dioxide (mmHg). A trained and certified sports scientist continuously supervises the examination, and a physician is always available on request when testing CHF patients and participants older than 50 years. In the absence of chest pain and electrocardiogram (ECG) abnormalities, all tests will be continued until maximal exertion (i.e., volitional exertion, dyspnea, or fatigue). The capillary blood lactate concentration from the earlobe will be measured at rest, at maximum performance, and at one and three minutes after the end of the exercise test. The Borg scale [91] will be applied during warm-up and every two minutes thereafter until exhaustion. Before and during the test, patients will be encouraged to reach maximal exhaustion. All tests will be performed according to the current guidelines for exercise testing and in controlled humidity and temperature conditions [92]. Before each test, the equipment will be calibrated in standard fashion with reference gas and volume calibration. A standard 12-lead ECG will be obtained at rest, during the entire period of the exercise test, and for three minutes during recovery. The ECG will be equipped with an analysis of high-frequency components of QRS complexes (HFQRS) to improve the diagnostic value of exercise ECG [93].

$\dot{V}E$ (L/min), $\dot{V}O_2$ (mL/min), and $\dot{V}CO_2$ (mL/min) will be acquired on a breath-by-breath basis and averaged over 10-s intervals. $\dot{V}O_2$peak will be defined as the highest 30-s average of $\dot{V}O_2$ at any point of the test. The $\dot{V}E$/maximal voluntary ventilation (MVV) will be calculated as peak $\dot{V}E$ in relation to MVV, and MVV will be calculated as forced expiratory volume in one second (FEV1) multiplied by 40 [92].

Blood pressure will be measured manually during warm up, every two minutes during the ramp protocol, immediately before maximal exertion, and during the recovery phase. Arterial oxygen saturation will be recorded continuously using a pulse oximeter (Masimo Corporation, Irvine, CA, USA).

Blood lactate concentration in mmol/L will be measured from 10 μl capillary blood drawn from the ear. The analysis of blood lactate concentrations will be done via the SuperGL Ambulance (Hitado Diagnostic Systems, Moehnesee, Germany) immediately after the last blood sample is drawn.

Muscle strength: isometric leg strength, countermovement jump, and grip strength

Isometric leg strength will be measured in both legs simultaneously, using an analogue dynamometer (TTM Muscular Meter, Tokyo, Japan). Participants will be instructed to lift the bar upward with maximum force [94] using only their legs and keeping their back straight. The test will be performed at a knee angle of 110°. This test examines isometric strength, predominantly of the quadriceps and hip extensors.

The countermovement jump will be performed on a force plate (Leonardo Mechanograph®, Novotec Medical, Pforzheim, Germany) to measure peak power. The instruction will be to jump with the head and chest as high as possible, thus producing the maximum elevation of the centre of mass. Participants who are unable to jump will be instructed to push as fast and hard as possible in order to generate power on the plate. This procedure can be performed by any participant without security concerns. Each participant will perform three trials. The most critical outcome parameter of this test is the maximum power output (peak power) normalized to the body weight of the participant. The method has been validated in young and older adults [95].

Hand grip strength will be measured using a handheld dynamometer (Leonardo Mechanograph GF; Novotec Medical GmBH, Pforzheim, Germany). Participants will perform the test standing, using the dominant hand. Three attempts will be performed with participants in a standing position with their elbow in full extension, resting for 60 s between attempts [96]. Grip span will be adapted to the individual hand size [97]. Maximal achieved grip strength (kg), and rate of force development (RFD) will be used for the analyses. RFD describes the capacity to produce voluntary activation in the early phase of contraction (first 75 ms) [98],

Neuromuscular coordination: balance test and gait analysis

A force plate (Leonardo Mechanograph®; Novotec Medical, Pforzheim, Germany) will be used to assess the centre of pressure during an upright static tandem stance. Patients will be asked to maintain an upright position with their knees slightly flexed (~ 10°), hands at their side, and their gaze straight ahead for 10 s on a cross 1.5 m away on the wall. The cumulative sway path during this period will be registered and serves as a measure of postural control. To minimize bias through potential learning effects, the test will be repeated three times. Additionally, failed attempts will be recorded should

a patient seek support [99]. This test has been shown to have a good degree of reliability [100].

An inertial sensor system (Physilog®; GaitUp, Lausanne, Switzerland) will be used to analyse participants' gait. The lightweight sensors, integrating a three-axis accelerometer and a gyroscope, will be attached to the participants' feet. Participants will be asked to walk at their habitual speed on a 20-m walkway (two-way, one attempt). The sensor system has previously shown good validity and reliability in assessing speed, spatiotemporal parameters, and foot kinematics of walking [101, 102].

Vascular aging

Macrovascular measurements: arterial stiffness measurement and flow-mediated dilation

Arterial stiffness will be measured as baPWV using a noninvasive vascular screening system (VaSera VS-1500 N; Fukuda Denshi, Tokyo, Japan). The participants are requested to fast for at least two hours before the examination and to abstain from alcohol and caffeine on the day of the examination. After 10 min of rest in a quiet, dark room with ambient temperature (23 °C–26 °C) in a supine position, measurements will be performed. Blood cuffs will be placed above the left ankle and the left upper arm. A foot-to-foot method will be used to determine the time delay of the pulse wave from the heart to the ankle. Using a height-based formula, vascular length between the heart valve and the ankle artery will be estimated as baPWV by the VSS-30 software (Fukuda Denshi, Tokyo, Japan) [103]. In addition, the central PWV will be calculated by the application of the ARC-Solver algorithm to pulse wave signals acquired with the VaSera VS-1500 device to estimate central systolic blood pressure (cSBP) and aortic PWV [104]. Peripheral and central blood pressure, pulse wave reflection as augmentation index, and arterial stiffness are commonly used as independent predictors to assess CV risk [59].

The FMD examination follows the measurement of arterial stiffness after at least 15 to 20 min of rest in the supine position. An occlusion cuff will be wrapped around the right forearm with the proximal edge of the cuff at the elbow. Using a high-resolution ultrasound linear array transducer, longitudinal images of the right brachial artery (typically located at 3–15 cm above the elbow) are recorded at the baseline and after cuff deflation following suprasystolic compression (50 mmHg over the systolic blood pressure value) of the right forearm for five minutes, until three minutes after deflation. FMD is measured by the A-mode waves as a signal of the intima-media complex (Unex Corporation, Nagoya, Japan). Furthermore, blood flow velocity will be measured according to pulsed Doppler values at the baseline and during peak hyperaemic flow. Inter-reader and intersession reliability of the FMD measurement was shown to be acceptable [105, 106].

Microvascular retinal measurements: static retinal vessel analysis and dynamic retinal vessel analysis

Static retinal vessel diameters will be analysed at a second visit using the Dynamic Vessel Analyser (DVA; IMEDOS Systems, Jena, Germany) as previously described [107]. Twenty minutes after pupil dilatation, measurements of retinal arteriolar and venular diameters will be performed. Three valid images are taken from the retina of the right eye with an angle of 30° and with the optic disc in the centre, per visit. Retinal arterioles and venules, coursing through an area of 0.5 to 1 disc diameter from the optic disc margin, will be identified semiautomatically at higher magnification using special analysis software (Vesselmap 2; IMEDOS Systems, Jena, Germany). Diameters will be averaged to central retinal arteriolar and venular equivalents (CRAE and CRVE), using the Parr–Hubbard formula described elsewhere [108], and the AVR will be calculated from the CRAE and CRVE. The reliability of this method is high, with interobserver and intraobserver interclass correlation coefficients for arteriolar and venular diameter measurements ranging from 0.78 to 0.99 [108, 109].

Dynamic retinal vessel imaging will be performed in one eye with the same DVA device as previously described [107]. Arteriolar and venular vessel branches measuring approximately 1 mm in length, located in the upper temporal quadrant 1 to 2 optic disc diameters away from the optic disc edge, will be assessed. Retinal vessels will be stimulated with a flickering light relying on the principles of neurovascular coupling. The vascular stimulation and its underlying principles have been previously described [110]. In brief, an optoelectronic shutter is inserted into the retina camera in place of an additional optical filter. The shutter interrupts the observation light (530–600 nm) with a frequency of 12.5 Hz and provides a sequence of one normal illuminated and one dark single frame at a video frequency of 25 Hz. The measurement of the baseline vessel diameter for 50 s is followed by three cycles of 20 s of flicker provocation and 80 s of observation. The total duration of the measurements, including baseline and observations between flicker provocations, amount to 350 s.

Echocardiography

Echocardiography will be performed with the Fukuda UF 760 ultrasound scanner (Fukuda Denshi, Tokio, Japan) and with an SA16 (2–5 MHz) transducer (Fukuda Denshi, Tokio, Japan) by experienced physicians according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging [111]. In brief, echocardiography

includes the determination of EF (two-dimensional, modified Simpson rule), PW-Doppler E- and A-waves at the mitral wave; Tissue Doppler E´-, A´-, and S´-waves at the septal and lateral sites of the left chamber; and left atrial diameter (m-mode, parasternal long-axis view, and apical four- and two-chamber views). For the measurement of left ventricular mass, chamber dimensions and wall thicknesses will be acquired from the parasternal long- and short-axis views using targeted m-mode echocardiography at the level of the mitral valve leaflet tips at end diastole, with the m-mode cursor positioned perpendicular to the septum and the left ventricular posterior wall. All measurements will be analysed with a computerized review station (EZ Desk; Fukuda Denshi, Tokyo, Japan) as done in a previous study [60], with our lab as the core reading centre.

Intima-media thickness

Carotid intima-media thickness (CIMT) will be measured with the Fukuda UF 760 ultrasound scanner (Fukuda Denshi, Toyko, Japan) with a FUT-LA385-12P (8–13 MHz) transducer (Fukuda Denshi, Tokyo, Japan) according to standard procedures previously used in the SAPALDIA-cohort study [112]. Automatic measurements will be limited to the right common carotid artery to assess CIMT and carotid stiffness according to established procedures [113, 114].

Inflammation and circulating cardiovascular risk factors

Blood samples are drawn by venipuncture of the cubital fossa of the right or left arm by trained medical staff in fasting status (at least three hours). The total volume of blood samples taken are 2 × 2.7 mL potassium-EDTA, 2 × 7.5 mL serum-monovette, and 1 × 7.5 mL Li Heparin. Blood samples are immediately centrifuged, and the plasma aliquots are frozen at a temperature of − 80 °C. Planned blood analysis for basic characterization of risk factor profile include total cholesterol, low- and high-density lipoprotein, triglycerides (colorimetric tests), and haemoglobin A1c. N-terminal pro-hormone B-type natriuretic peptide will be analysed by standard laboratory assays with the Cobas analyser (Cobas 8000; Roche Diagnostics, Basel, Switzerland).

Anthropometry and questionnaires

All questionnaires will be digitally recorded. The standardized questionnaires used in this study are the following: the European Health Interview Survey-Physical Activity Questionnaire (EHIS-PAQ) [84], Global Physical Activity Questionnaire (GPAQ) [85], Physical Activity Readiness Questionnaire (PAR-Q), Munich Chronotype Questionnaire (MCTQ) [82], Health-related Quality of Life questionnaire, short form (SF-8) [83], Perceived Stress Scale (PSS) [87], Insomnia Severity Index (ISI) [88] and a shortened version of the University of Alabama at Birmingham (UAB) Study of Aging Life-Space Assessment [86]. General health and medical conditions, use of a walking aid, frequency of falls (12-month recall) [115], medication use, alcohol consumption, menstruation cycle, residential area, use of transportation, and socioeconomic status will also be assessed by self-report.

Body composition will be analysed by four-segment bioelectrical impedance analysis using the InBody 720 (Inbody Co. Ltd., Seoul, South Korea). Measurement of appendicular muscle mass with InBody 720 is acceptable as compared with dual-energy x-ray absorptiometry analysis [116, 117]. Participants will refrain from any intense physical activity for 24 h prior to measurement, will fast for a minimum of two hours, and will be asked to void their bladder before the measurement.

Physical activity

Physical activity will be objectively measured over 14 days [118, 119] using a wrist-worn triaxial accelerometer (GeneActive Activinsights Ltd., Kimbolton, UK). GeneActive accelerometers have previously been validated [120]. The device will be attached to the participant's nondominant wrist and samples data at a frequency of 50 Hz. Participants will be asked to wear the device continuously during day and night in their free-living conditions. Accelerometry data will be exported using the GENEActiv software version 2.9 (GENEActiv Activinsights Ltd., Kimbolton, UK) and will be collapsed into 60-s epoch files.

Statistical analysis

Participant characteristics will be analysed descriptively. The distribution of continuous variables will be inspected graphically and characterized with either the mean and SD or with the median and interquartile range. Categorical variables will be presented as absolute and relative frequencies. Age trajectories of components of physical fitness (endurance capacity, muscular strength, and neuromuscular coordination) will be analysed over decades, using multiple linear regression models [121]. Model diagnostics will include residual diagnostics and assessment of multicollinearity [122]. If necessary, standard errors, confidence intervals, and p-values will be adjusted for heteroscedasticity [123]. Model selection will be done based on the Akaike Information Criterion (AIC) [124]. Models will be adjusted for potential confounders such as body mass index, blood pressure, and sex. Both linear and nonlinear trajectories will be considered [125]. Sensitivity analyses will include the inclusion of interaction terms with the decade term to detect potential differences between subgroups of trajectories of components of physical fitness. Multiple imputation using chained equations (MICE) will

be used for variables with a high proportion of missing data [126]. Essential differences between multiple imputation analyses and complete case analyses will be discussed. All statistical tests will be two-sided with a significance level of 5%.

Analyses with health distance

We will explore new avenues in computing the health distance (HD) for healthy individuals and heart failure patients based on a methodology designed initially for longitudinal studies applying it to cross-sectional data [127–129]. In their research, Arbeev et al. [128] describe the analogue procedure on the optimal versus realized trajectories of physiological dysregulation in aging organism and their relation to sex-specific mortality risk in the framework of a mathematical model of aging and mortality [130]. A recent research [131] further elaborated the approach applying it to data on onset of and survival from aging-related diseases and illustrated that such measures can potentially be used as a preclinical indicator of transition from healthy to unhealthy state. This approach measures physiological dysregulation based on deviations of multiple biomarker profiles from their 'reference' values. In the present study, this method (originating from [129]) will be applied to all components of physical fitness, to the vascular imaging biomarkers, and to the established traditional risk factors.

The HD is defined as the statistical (Mahalanobis) distance [132] constructed for the joint distribution of multiple biomarkers [129]: $HD(X_i) = \sqrt{(X_i - \overline{X})^T S^{-1} (X_i - \overline{X})}$.

where X_i is a vector of biomarkers measured in the individual i and $\overline{X}$ and S are the vector of means and the variance–covariance matrix, respectively, in some 'reference' population from which the distance is computed.

We will use the younger part (defined using different cut-off ages, see discussion in the paragraph preceding section *Sample size calculation*) of the healthy (C-Health) population as the 'reference' population. For this group, we will compute the means and variance–covariance matrix for the sets of measured quantitative biomarkers (see measurements), separately for females and males. Next, the health distances for the C-Health population and for 'cases' (i.e., age-matched patients with heart failure) will be computed using the above equation, with respective sex-specific means and variance–covariance matrices $\overline{X}$ and S taken from the 'reference' population, and the null hypothesis that the distances in these two groups are equal will be tested.

A baseline scenario will allow us to use all available quantitative biomarkers to define the health distance. We will also specify different 'domain-specific' subsets of biomarkers (e.g., those corresponding to endurance capacity, muscular strength, neuromuscular coordination, etc.) as well as separate biomarkers to identify which (sets of) biomarkers produce larger distances from the 'reference population.' As the approach assumes multivariate normality of the biomarkers, we will transform each biomarker, as necessary, using the appropriate transformation (e.g., the Box–Cox transformation) and then standardize them to a zero mean and unit variance to ensure that all transformed biomarkers are on the same scale in the analyses. The approach also assumes there are no missing data; however, in case of missing data for some biomarkers, we will perform the analyses imputing missing values of biomarkers using a fully conditional specification method of multiple imputation [133], and apply the standard Rubin's rules for statistical inference.

We also note that many biomarkers (e.g., blood pressure, cholesterol, etc.) change nonlinearly with age, as shown in prior publications [134–137] that found a 'bell-shaped' relationship of the biomarkers with age (i.e., increasing and then declining). As a result, some biomarkers at the oldest ages (90–100 years) may be closer to the 'reference' values in the middle ages versus the values in the old ages (e.g., 70 years). Therefore, the relationship between the dynamics of biomarkers and aging is very complex and nonlinear (see, e.g., extensive discussion on the topic in a recent review paper [127]). The COmPLETE study, where biomarkers will be measured for individuals in broad age ranges spanning decades, provides extensive opportunities to check the possible nonlinearity of the patterns of the health distance with age. Although this is a cross-sectional study, it is an important contribution to this area of research, as this topic is still largely underexplored [127]. In this project, we will compute the health distances using different age groups to define the 'reference' populations and compare the health distances computed for different 'reference' groups (or for different decades of age for the same 'reference' group) for different combinations of biomarkers to tackle the question of the complexity of age-related changes in biomarkers and health.

Sample size calculation

We used simulations to estimate the required sample size for a linear regression model assessing the trajectory of $\dot{V}O_2$peak in seven 10-year decades [138]. Based on prior studies in healthy and active individuals, the standard deviation of the $\dot{V}O_2$peak was assumed to be 6.0 mL/kg/min for each decade [139–141]. Assuming a mean decrease of 5.0 mL/kg/min per decade [139–141], 34 participants per sex were determined to be needed to achieve the required power of 80% ($p = 0.05$). Additionally, 17 men and 17 women per decade were deemed

required to detect a difference between men and women regarding the trajectories of $\dot{V}O_2$peak.

We also conducted power analyses for the studies with the health distance assuming that the distribution (standard deviation = 1.077) and the dynamics (the annual rate of change = 0.034) of this measure resemble those in the Framingham data [128]. With the proposed sample size of 80 heart failure patients and 210 (70 participants per the decades 50–59, 60–69, and 70–79) age-matched healthy participants, the study will have the power of 80% to detect the difference between the health distances in these two groups of about 0.4 units for the two-sided test (about 0.35 units for the one-sided test). The Framingham data confirm that such a difference would correspond to differences between health distances for individuals who are about 12 (SD: 10) years of age apart.

Potential pitfalls and limitations

Pitfalls

The recruitment of 70 healthy individuals in the category of 80 years and older for C-Health and 80 participants with heart failure for C-Heart constitutes a challenge.

Limitations

We are aware that our study has some limitations. It is clear that the data acquired from C-Health will not be fully representative of the Swiss population, since we will only examine a circumscribed population sample from the Basel region. Selection bias through our recruitment strategy, considering an inclusion rate of 3 to 5% of invitations sent, cannot be excluded and it is likely that our study participants will have an improved physical function and a better health status versus individuals who received an invitation but did not take part in the study. However, since we are not trying to recruit a representative sample of Swiss or Basel citizens but rather a sample of healthy male and female across the age groups, we think potential selection bias is a negligible problem for our aims.

Selection bias, however, might become more evident with ascending age decades in C-Health. A negative correlation between physical activity and physical fitness and some exclusion criteria such as high blood pressure, a body mass index of ≥30 kg/m^2, or chronic disease can be assumed. The individuals meeting the inclusion criteria might, therefore, be more fit and active than their age groups members not meeting the inclusion criteria. This effect could be more significant in the older age groups and decrease the effect of age on the measured physical fitness parameters. However, a goal of our study is to describe the reduction in fitness markers only due to aging and not a chronic disease; this potential limitation could also be seen as a strength.

Further limitations are circadian, seasonal, and hemodynamic fluctuations, which influence vascular as well as physical fitness markers. The exact time of day and the season will be recorded to control for these fluctuations.

Further, there are some limitations concerning our measurement methods. We do not use the gold standard measurement for the assessment of isometric leg strength, which would either be by an isokinetic dynamometer or a custom-built isometric strength testing chair with an external analogue-to-digital converter. Further, we decided to use only one balance task. The tandem stance could be too easy for young individuals and cumulative change in sway paths may not discriminate between good and poor neuromuscular balance. On the other hand, this task could already overburden older participants who might be unable to perform the tandem stance for 10 s without seeking support.

We are aware that this is a cross-sectional observational study, and reverse causation is possible. However, no previous data exist regarding different comprehensive components of physical fitness, and potential follow-up in five years may allow for strengthening of the findings.

Discussion

The COmPLETE study with its design will allow for the investigation and characterisation of the physical fitness components of endurance capacity, muscle strength, and neuromuscular coordination in individuals without chronic diseases from the 20th to the 100th year of life as well as in patients with heart failure. Therefore, this study will construct a novel dataset with normal values for all major physical fitness markers in healthy individuals.

The additional comprehensive assessment of vascular biomarkers in these individuals offers the opportunity to discriminate within apparently healthy individuals. Separately, it allows for the investigation of the mechanisms of aging and the role of physical fitness components and physical activity on vascular markers in various vasculature beds in health and heart failure.

Furthermore, the COmPLETE study may elucidate new approaches in diagnosis with its combined and extensive assessment of physical fitness and vascular biomarkers. This will enable us to find the most suitable diagnostic markers for CV risk and heart failure.

According to the inverse association of several vascular biomarkers with physical fitness components (endurance capacity, muscle strength, and neuromuscular coordination), individuals with excellent vascular health markers may have even better physical fitness than those with attenuated vascular health within the C-Health sample.

The age-matched comparison with patients with different stages of heart failure may provide an estimate of the health distance of different fitness parameters to healthy individuals.

Health distance provides a new complex measure of aging-related decline in the adaptive capacity of the organism by comparing the values of the physiological or biological "norms" (C-Health) with those with prevalent heart failure (our example) [128].

The COmPLETE study shall provide a better understanding of which functional characteristics should be specifically targeted in primary and secondary prevention to achieve an optimal healthspan.

Abbreviations

AIC: Akaike information criterion; AVR: Arteriolar-to-venular diameter ratio; baPWV: Brachial–ankle pulse wave velocity; BNP: B-type natriuretic peptide; C-Health: COmPLETE-Health; C-Heart: COmPLETE-Heart; CHF: Chronic heart failure; CIMT: Carotid intima-media thickness; CPET: Cardiopulmonary exercise testing; CRAE: Central retinal arteriolar equivalents; CRVE: Central retinal venular equivalents; cSBP: Central systolic blood pressure; CV: Cardiovascular; DVA: Dynamic vessel analyser; EF: Ejection fraction; EOV: Exercise oscillatory ventilation; FEV1: Forced expiratory volume in one second; FMD: Flow-mediated dilation; HD: Health distance; HFmEF: Heart failure with mid-range ejection fraction; HFpEF: Heart failure with preserved ejection fraction; HFQRS: High-frequency components of QRS complexes; HFrEF: Heart failure with reduced ejection fraction; LVEF: Left ventricular ejection fraction; MICE: Multiple imputation using chained equations; MVV: Maximal voluntary ventilation; NYHA: New York heart association; OUES: Oxygen uptake efficiency slope; PETCO2: Partial pressure of end-tidal CO2; PWV: Pulse wave velocity; RFD: Rate of force development; SCORE: Systematic coronary risk evaluation; SD: Standard deviation; $\dot{V}E/\dot{V}CO_2$ slope: Relationship of the ventilation to carbon dioxide production slope; $\dot{V}O_2$peak: Peak oxygen uptake

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Authors' contributions

Conceptualization, AST, JW, RK and TH; Methodology, JW, RK, TH, AST, HH, RR, OF; Formal Analysis, DI and KA; Investigation, JW and RK; Resources, HH, TH, OF, RR; Data curation, RK and JW; Writing – Original Draft, JW and AST; Writing – Review & Editing, RK, HH and TH; Supervision, HH, TH, MB, TD; Project Administration, JW and RK; Funding Acquisition, AST. All authors read and approved the final manuscript.

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Availability of data and materials

not applicable.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017–01451) and complied with the declaration of Helsinki. Written informed consent was obtained from all study participants.

Consent for publication

not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Sport, Exercise and Health, University of Basel, Birsstrasse 320 B, 4052 Basel, Switzerland. [2]Biodemography of Aging Research Unit (BARU), Social Science Research Institute, Duke University, Durham, NC 27705, USA. [3]Department of Clinical Research, Basel Institute for Clinical Epidemiology and Biostatistics, University Hospital Basel, Spitalstrasse 12, 4056 Basel, Switzerland. [4]Department of Health Research Methods, Evidence, and Impact, McMaster University, 1280 Main Street, Hamilton ONL8S4K1, Canada. [5]University Clinic of Medicine, Cantonal Hospital Baselland, University of Basel, Rheinstrasse 26, 4410 Liestal, Switzerland.

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Publisher's Note

Chapter 5

Publication 3

New Data-based Cutoffs for Maximal Exercise Criteria Across the Lifespan.

Authors:

Jonathan Wagner[1], Max Niemeyer[2], Denis Infanger[1], Lukas Streese[1], Henner Hanssen[1], Jonathan Myers[3], Arno Schmidt-Trucksäss[1]*, Raphael Knaier[1]*

[1] Department of Sport, Exercise and Health, Division Sports and Exercise Medicine, University of Basel, Basel, Switzerland

[2] Department Medicine, Training and Health, Institute of Sport Science and Motology, Philipps-University Marburg, Marburg, Germany

[3] Cardiology Division, VA Palo Alto Health Care System and Stanford University, Palo Alto, CA, USA

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Chapter 6

Publication 4

Novel CPET Reference Values in Healthy Adults: Associations with Physical Activity.

Authors:

Jonathan Wagner[1], Raphael Knaier[1], Denis Infanger[1], Karsten Königstein[1], Christopher Klenk[1]
Justin Carrard[1], Henner Hanssen[1], Timo Hinrichs[1], Douglas Seals[2], Arno Schmidt-Trucksäss[1]

[1] Department of Sports, Exercise and Health, University of Basel, Basel, Switzerland.
[2] Department of Integrative Physiology, University of Colorado Boulder, CO, USA.

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Abstract

Purpose

Cardiopulmonary exercise testing (CPET) is an important measurement in clinical practice and its primary outcome, maximal oxygen uptake ($\dot{V}O_{2peak}$), is inversely associated with morbidity and mortality. The purpose of this study is to provide CPET reference values for maximal and submaximal parameters across the adult age spectrum of a healthy European cohort, to compare $\dot{V}O_{2peak}$ values with other reference datasets and to analyze the associations between physical activity (PA) levels and CPET parameters.

Methods

In this cross-sectional study, we prospectively recruited 502 participants (47% female) from 20 to 90 years old. The subjects performed a CPET on a cycle ergometer using a ramp protocol. PA was objectively and continuously measured over 14 days using a triaxial accelerometer. Quantile curves were calculated for CPET parameters. To investigate the associations between CPET parameters and PA levels, linear regression analysis was performed.

Results

$\dot{V}O_{2peak}$ values observed in the group of 20-29 years were 46.6±7.9 and 39.3±6.5 (mL/kg/min) for males and females, respectively. On average, each age category (10-year increments) showed a 10% lower $\dot{V}O_{2peak}$ relative to the next younger age category. $\dot{V}O_{2peak}$ values of previous studies were, on average 7.5 (mL/kg/min) (20%) lower for males and 6.5 (mL/kg/min) (21%) lower for females. There was strong evidence supporting a positive association between $\dot{V}O_{2peak}$ (mL/kg/min) and the level of habitual PA performed at vigorous PA (estimate 0.26; $p < 0.001$].

Conclusion

Maximal and submaximal CPET reference values over a large age range are novel and differences to other studies are clinically highly relevant. Objectively measured vigorous-intensity PA showed a strong positive association with higher $\dot{V}O_{2peak}$ and other performance-related CPET parameters, supporting the implementation of higher intensity aerobic exercise in health promotion.

Introduction

Cardiopulmonary exercise testing (CPET) is an important clinical assessment as described in a recent statement by the American Heart Association and its primary outcome, $\dot{V}O_{2peak}$, should be assessed as a clinical vital sign (1). Reduced $\dot{V}O_{2peak}$ is a very strong risk factor and inversely associated with morbidity and mortality (2, 3).

The first $\dot{V}O_{2peak}$ reference values were made available decades ago (4) and, since then, many more have been published (5). However, the available $\dot{V}O_{2peak}$ reference values (6, 7) differ largely from one another, which can mainly be explained by variations among the studied populations, exercise modes, exhaustion criteria used, and subjects' physical activity (PA) levels. $\dot{V}O_{2peak}$ and other CPET parameters seem to be highly dependent upon the recruitment and selection of the studied population. Many of the reference values have included smokers, subjects with obesity, cancer, diabetes, or other chronic diseases and cardiovascular risk factors (5). Therefore, a large initiative supported by the American Heart Association, the FRIEND registry called for the need to develop accurate $\dot{V}O_{2peak}$ reference values on a global scale (8, 9). Further, many studies lack reports of submaximal CPET markers. Beyond $\dot{V}O_{2peak}$, CPET has revealed other valid markers to predict health outcomes in both healthy individuals and patients with heart failure such as $\dot{V}E/\dot{V}CO_2$ slope, oxygen uptake efficiency slope (OUES), and partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) at rest and at ventilatory threshold 1 (VT1) (10). Combined with $\dot{V}O_{2peak}$, these secondary outcomes can improve guidance of clinical decision-making, PA recommendations, specific exercise interventions, medical therapy, and surgical interventions (1, 10). Therefore, reporting reference values for secondary outcomes in addition to $\dot{V}O_{2peak}$ is essential, but often neglected. As such, rather than adopting a big data approach, this prospectively planned study focused on ensuring rigorous data assessment, strict exclusion criteria for diseases or health risk factors limiting aerobic exercise capacity, fulfilling high secondary exhaustion criteria, assessing subjects' PA levels objectively by accelerometry and reporting secondary outcomes. For an optimal interpretation of CPET parameters, knowledge of the physiological responses and variations among healthy individuals are decisive. Such reference values have the potential to increase the sensitivity and specificity of CPET parameters to differentiate pathological responses to CPET. The COmPLETE-Health Study was planned in accordance with the available guidance for an optimal set of normal values offered by the American Thoracic Society/American College of Chest Physicians (11) and meets at least 12 of the 14 listed criteria described in a systematic review by Takken et al. (5). Specifically, the COmPLETE-Health Study is the first study (12, 13) reporting CPET parameters using a cycle ergometer and fulfilling almost all criteria.

In addition to reporting PA levels to characterize the studied sample according to ATS/ACCP guidelines, its association with CPET parameters shall be quantified. Even though previous studies have found that habitual PA and $\dot{V}O_{2peak}$ are only modestly correlated at the population level (14, 15), PA programs remain the main intervention strategy to increase $\dot{V}O_{2peak}$. When looking at

intensity levels at which PA is performed, several randomized trials have previously suggested that a greater benefit of vigorous-intensity exercise exists relative to low- and moderate-intensity exercise (16). To date, however, no population-based study has examined the association of objectively measured PA and cardiorespiratory fitness (determined by respiratory gas measurement) across an age range from 20 to 90 years.

This study has several aims. First, it adds prospectively assessed new reference values for $\dot{V}O_{2peak}$ and additional CPET markers of a healthy White European cohort by using standardized percentile tables. Second, $\dot{V}O_{2peak}$ values are compared with the major CPET cycle ergometer reference datasets available to show the impact of the sample's health status on $\dot{V}O_{2peak}$ values. Third, the associations of objectively assessed light PA (LPA), moderate PA (MPA), and vigorous PA (VPA) with submaximal and maximal CPET parameters is analyzed.

Methods

Population and recruitment

All participants were recruited from the Basel area, Switzerland, including approximately 35 males and 35 females per age category (i.e., 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80+ years of age). The adopted recruitment strategy involved invitation letters sent to randomly selected households. The exact recruitment scheme has been described in the study protocol published elsewhere (17). A telephone screening of potential participants was conducted before making appointments. Final eligibility was confirmed onsite on the day of examination. Eligible persons were healthy men and women aged between 20 and 100 years with a body mass index of less than 30 kg/m^2 and being a permanent nonsmoker or ex-smoker for at least 10 years. Exclusion criteria included any kind of manifest exercise limiting chronic diseases such as myocardial infarction, stroke, heart failure, lower-extremity artery disease, any kind of diagnosed cancer, diabetes, clinically apparent renal failure, severe liver disease, chronic bronchitis GOLD stages II to IV, osteoporosis, known pregnancy or breastfeeding, drug or alcohol abuse, blood pressure ≥ 160/100 mmHg, exercise compromising orthopedic problems, clinical apparent Alzheimer's disease, or any other form of dementia.

Setting

This study was carried out at the Department of Sport, Exercise, and Health at the University of Basel, Switzerland, and was funded by the Swiss National Science Foundation (grant no. 182815). It was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451). Written informed consent was obtained from all study participants prior to inclusion. This study is registered at www.clinicaltrials.gov NCT03986892.

Participants of the COmPLETE-Health Study underwent tests lasting approximately 3.5 to 4 hours including the CPET. PA was monitored over 14 days by a wrist-worn accelerometer following

the laboratory testing. The precise sequence of the various measurements is described in detail in the study protocol (17).

Acquisition of participants` characteristics

Height and body weight were measured to the nearest 0.5 cm and 0.1 kg, respectively, and the body mass index was calculated. A four-segment bioelectrical impedance analysis was conducted (Inbody 720; Inbody Co. Ltd., Seoul, South Korea) to measure body fat content and lean body mass. Resting systolic and diastolic blood pressures and heart rate were measured with the participant in the supine position using a non-invasive vascular screening system (VaSera VS-1500 N; Fukuda Denshi, Tokyo, Japan). Smoking status was assessed by telephone interview prior to the appointment, while physicians reviewed medical history and medications by questionnaire onsite. Further, a 12-lead resting electrocardiogram was acquired and reviewed by a physician immediately before the exercise test. Blood samples were drawn by venipuncture by trained medical staff in fasting status (at least three hours, mean five hours). Blood samples were immediately centrifuged, and the plasma aliquots were frozen at a temperature of −80 °C. Blood parameters for basic characterization of risk factor include total cholesterol, low- and high-density lipoprotein, triglycerides and HbA1c.

Cardiopulmonary exercise testing

An exercise test to maximal voluntary exertion using an electromagnetically braked cycle ergometer (Ergoselect 200; Ergoline, Bitz, Germany) was performed according to one of the following five ramp protocols: i) a three-minute warm-up either unloaded, a load of 10 or 20 W for protocols 1 to 3, or a load of 50 W for protocols 4 and 5 followed by ii) a ramp protocol with a linear workload increases of 7, 10, 15, 20, or 30 W/min for protocols 1 to 5 respectively. The protocol was chosen to achieve a ramp duration of 10 minutes and measurements were excluded when the exercise time was not between six and 18 minutes. Pedaling cadence was chosen by participants but was required to be more than 60 revolutions per minute.

Gas exchange and ventilatory variables were analyzed continuously (breath-by-breath) using a computer-based system (MetaMax 3B; Cortex Biophysik GmbH, Leipzig, Germany). Every test was preceded by a resting period of three minutes to reach steady-state conditions. A trained and certified sports scientist continuously supervised the examination, and a physician was always available on request. In the absence of chest pain and electrocardiogram abnormalities, all tests were continued until maximal exertion (i.e., volitional exertion, dyspnea, or fatigue). Blood lactate concentration in mmol/L was analyzed from 10 µL of capillary blood drawn from the earlobe measured at rest, at maximum performance, and at one and three minutes after the end of the exercise test. The analysis of blood lactate concentrations was performed via the SuperGL Ambulance (Hitado Diagnostic Systems, Moehnesee, Germany) immediately after the last sample was drawn. Before and during the test, patients were encouraged to reach their level of maximal exhaustion. All tests were

performed in controlled humidity and temperature conditions (11). Before each test, the equipment was set up in standard fashion with reference gas and volume calibrations. $\dot{V}O_{2peak}$ was defined as the highest 30-second average of $\dot{V}O_2$ at any point during the test. Only a small number of well-trained exercise physiologists performed and supervised the CPETs, and standardized procedures and instructions were applied to ensure equal testing conditions for all participants. The following exhaustion criteria needed to be reached by the participants in order for them to be included in the final analysis: for the participants aged 20 to 39 years, a respiratory exchange ratio (RER) of 1.13 or greater; for those aged 40 to 59 years, a RER of 1.10 or greater; and, for those aged 60 to 69 years, a RER of 1.06 or greater (18). For participants > 70 years the use of secondary exhaustion criteria is not recommended (18), due to the large inter-individual differences reported in this age group. However, in this study sample the $\dot{V}O_2$-plateau incidence (18) in individuals aged 70+ years did not differ from any age group under 70 years, suggesting that older individuals were exhausted to the same extend.

Physical activity

PA was objectively measured continuously over 14 days using a wrist-worn triaxial accelerometer (GeneActive Activinsights Ltd., Kimbolton, UK). The device was attached to the participant's nondominant wrist and sampled data at a frequency of 50 Hz. Participants were asked to wear the device continuously throughout the day and night in their free-living conditions. Accelerometry data were exported using the GENEActiv software version 3.2 (GeneActive Activinsights Ltd., Kimbolton, UK) and were collapsed into 60-second epoch files. The validated open-source Excel macro file 'General physical activity' version 2 (Activinsights Ltd., Kimbolton, UK) (19) was used to analyze the data. A valid day was defined as 10 hours or more of diurnal wear-time, up to 1,080 minutes of LPA, up to 480 minutes of MPA, and less than 150 minutes of VPA. To be included in the analyses, at least five weekdays and two weekend days of valid accelerometry data were required (20). The numbers of minutes per day performed at light PA (LPA) (1.5–3.99 METS), MPA (4.00–6.99 METS), and VPA (≥ 7 METS) were averaged for all valid days.

Statistical analysis

Participant characteristics were analyzed descriptively. The distribution of continuous variables was inspected graphically and characterized by either means and standard deviations or with medians and interquartile ranges. Categorical variables are presented as absolute and relative frequencies. Age- and sex-specific quantile curves were calculated using generalized additive models for location, scale, and shape (GAMLSS, R package version 5.1-6) (21). The age-trajectories were modeled using P-splines. We adopted the Bayesian information criterion to select the conditional distribution that offered the best compromise between model complexity and goodness-of-fit. The model fits were inspected using diagnostic residual plots such as worm plots (22) and Q–Q plots. Empirical data are reported in addition to the model-based quantiles and were

chosen for comparison with those of other studies. To investigate the associations between CPET parameters and PA levels, linear regression analysis with the respective CPET parameters as dependent variables and age, sex, and the activity variables as independent variables was performed. The activity variables (LPA, MPA, and VPA) were included all together in the same model. We modeled age using restricted cubic splines (natural splines) with four knots included along with an interaction by sex to control for the nonlinear age progression (23). To check whether the relationship between the three activity variables and CPET parameters differs between sexes (interaction), two separate models were calculated, including one with three interaction terms between sex and activity variables and one without any interactions. The two nested models were then compared using likelihood ratio tests to check whether the interactions improved the model fit substantially. There was little evidence for any CPET parameter suggesting that the interactions were necessary; therefore, they were omitted. For the PA variables, only linear relationships with the CPET parameters were checked. For some models, the residuals exhibited heteroskedasticity. Therefore, robust *p*-values and confidence intervals were calculated for all models (HC3) (24). No relevant multicollinearity between the activity variables could be found. A mediation analysis was employed to investigate whether PA variables acted as mediator of the associations between age and $\dot{V}O_{2peak}$. To quantify direct and indirect effects of PA variables, we used a structural equation model (SEM). The SEM assumed a linear association between age and $\dot{V}O_{2peak}$ and was adjusted for sex. R version 3.6.1 or later (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses and *p*-values ≤ 0.05 were considered statistically significant. All tests were two-sided.

Results

A total of 629 subjects took part in the COmPLETE-Health Study (Figure 1). In the final analysis, the CPET data of 502 participants (264 males and 238 females) were included after excluding participants with missing CPET results, with insufficient exercise test durations, test outcomes that did not pass the data-validity check, and after applying the exhaustion criteria. Participants were equally distributed across age decades from 20 to older than 80 years, with at least 66 participants representing every decade. Participant characteristics from medical examinations, blood testing, and PA levels are presented, by sex and 10-year age groups, in Table 1. The participants showed a healthy metabolic profile with low cardiovascular risk (Table 1, (25)).

Figure 1: *Flowchart.*

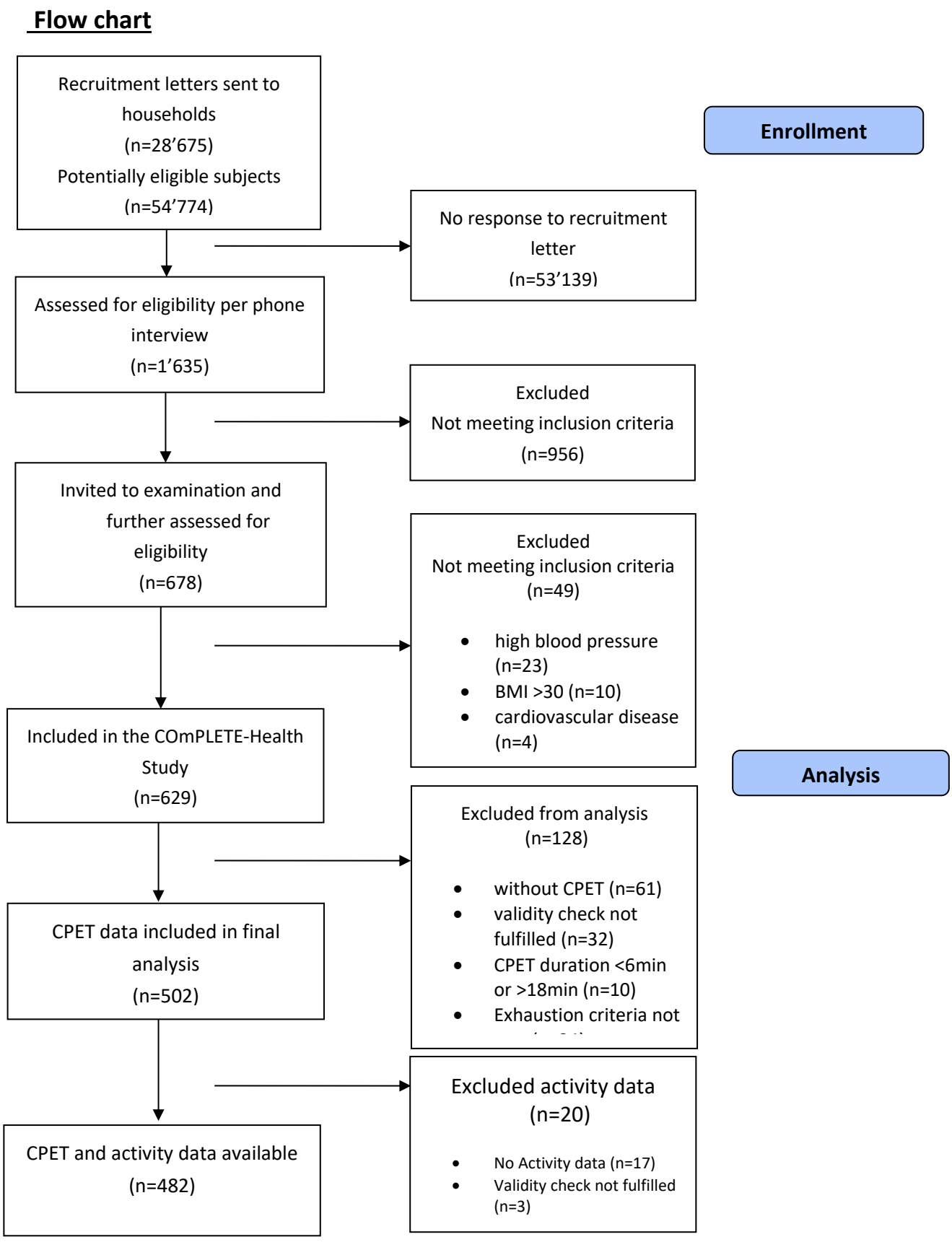

Table 1.*Descriptive characteristics of the study population separated by sex. Data are presented as mean ± standard deviation if not stated otherwise.*

	20-29		30-39		40-49		50-59		60-69		70-79		80+	
	male	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Participants, no. (%)	37 (7.4)	30 (6.0)	40 (8.0)	33 (6.6)	36 (7.2)	35 (7.0)	35 (7.0)	35 (7.0)	40 (8.0)	36 (7.2)	41 (8.2)	38 (7.6)	35 (7.0)	31 (6.2)
Age (yr)	24.9±2.7	24.7±2.3	34±3	33.7±3	45.4±3	44.7±3.3	54.8±3	54.6±2.9	64±2.8	65.2±3	73.9±2.5	74±2.3	83.2±3.1	84.1±2.3
Height (cm)	179.5±5.5	167.4±6.2	180.1±6.9	168.9±5.1	179.2±5.8	169±6	179.7±7	166.6±5.9	176.6±6.1	162.3±6.1	173.9±7.2	162±6.7	172.1±6.4	160±5.7
Body mass (kg)	76.7±9.5	60.7±9	76.9±9.6	62.8±9.2	77.1±9.2	65.6±7.7	81±9.8	62.7±8.1	76.6±9.6	60.9±6.6	75.2±8.2	61.4±7.8	76.3±9.3	58.9±8.8
BMI (kg/m^2)	23.8±2.3	21.8±3.1	23.7±2.3	22±2.9	24±2.3	23±2.4	25±2.2	22.6±2.4	24.5±2.4	23.1±2.4	24.9±2.6	23.4±3	25.7±2.8	23±2.8
Body fat content (%)	15±5	24±7	16±5	24±7	17±7	24±7	21±5	27±5	20±6	30±6	24±6	30±8	27±6	33±7
Lean bodymass (kg)	64.9±7.7	45.7±4.4	64.6±7.1	47.3±3.8	63.9±6.8	49.3±4.7	64.2±7.7	45.6±4.6	61±5.9	42.2±4.1	56.5±5.2	42.7±4.1	55.4±5	39.5±4.5
Rest systolic BP, mmHg	126±11	112±7	127±10	115±12	124±11	117±9	129±11	121±10	134±12	129±14	133±13	137±13	135±17	135±14
Rest diastolic BP, mmHg	71±7	70±7	76±7	71±9	77±8	71±8	82±7	77±8	84±7	81±8	81±9	79±8	82±10	79±7
HR at rest (bpm)	60±11	62±9	63±11	62±10	57±8	59±9	61±11	63±9	59±8	64±13	60±8	64±7	64±14	66±9
Smoking Status, n (%)														
Never smoked (%)	36 (97)	29 (97)	36 (90)	29 (88)	30 (83)	27 (77)	30 (86)	25 (71)	30 (75)	25 (69)	22 (54)	27 (71)	26 (74)	27 (87)
Ex-smokers >10 yr (%)	1 (3)	1 (3)	4 (10)	4 (12)	6 (17)	8 (23)	5 (14)	10 (29)	10 (25)	11 (31)	19 (46)	11 (29)	9 (26)	4 (13)
Medication, n (%)														
Antihypertensives (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6)	2 (6)	6 (15)	4 (11)	10 (24)	3 (8)	11 (31)	9 (29)
of which ACE ARB (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6)	2 (6)	4 (10)	3 (8)	10 (24)	2 (5)	10 (29)	9 (29)
Beta-blockers (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.5)	2 (6)	2 (5)	1 (3)	4 (11)	2 (6)
Anticoagulants (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	2 (6)	3 (7)	2 (5)	7 (20)	5 (16)
Statins (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	1 (3)	5 (12)	3 (8)	4 (11)	4 (13)
Blood testing, mg/dL														
HbA1c (mg/dL)	5.0±0.2	5.1±0.2	5.0±0.3	5.2±0.3	5.1±0.3	5.1±0.4	5.1±0.4	5.0±0.4	5.2±0.3	5.3±0.3	5.3±0.3	5.4±0.3	5.6±0.3	5.7±0.3
LDL cholesterol (mg/dL)	97.1±22.1	100.8±18.7	109.5±21.9	103±23.2	119.8±22	115.5±21.1	134.5±22.1	137.8±26	130.9±23.9	145.5±25.9	131.7±27.8	138.1±22.9	121.2±27.3	132.7±29.3
HDL cholesterol (mg/dL)	54.8±9.7	67.9±15.3	55±10.3	71.4±14.8	59.5±13.6	70±11.9	59.3±10.1	74.2±15.5	60.2±14.5	75.2±13.4	61.4±12.8	74.4±13.8	63.7±11.8	74.2±15.4
Total cholesterol (mg/dL)	180±38	190±31	199±33	193±36	213±34	209±30	229±32	239±37	227±35	253±37	230±43	252±32	221±41	246±41
Triglyceride (mg/dL)	120±74	100±47	146±90	80±36	126±78	99±39	137±64	94±33	135±56	124±57	120±39	125±92	109±35	133±65
Physical activity, min/day														
Light physical activity	85±21	90±19	95±31	98±24	86±25	113±35	87±28	106±25	97±31	118±33	97±30	116±31	95±26	121±34
Moderate physical activity	169±42	182±41	182±58	176±55	177±54	196±57	169±67	159±54	172±55	167±62	136±53	145±69	100±42	131±52
Vigorous physical activity	11±14	9±8	8±7	8±9	12±15	8±6	10±11	5±4	10±12	3±6	6±10	2±3	1±3	2±4

Abbreviations: BMI, body mass index; BP, blood pressure; HR, heart rate; ACE, angiotensin-converting-enzyme; ARB, AT1-receptor-blocker; LDL, low-density lipoprotein; HDL, high density lipoprotein.

CPET parameters

Maximal and submaximal CPET parameters are presented as empirical data in Table 2. The differences in relative $\dot{V}O_{2peak}$ (mL/kg/min) between two neighboring age decades were between −2% (−0.8 mL/kg/min) and −20% (−6.1 mL/kg/min) for males and between −1% (−0.4 mL/kg/min) and −18% (−5.6 mL/kg/min) for females, respectively. Larger differences were observed between older age categories and therefore the data describe a nonlinear decline. On average, each age category showed a 10% (90% of the previous value) lower $\dot{V}O_{2peak}$ (mL/min/kg) relative to the next younger age category. When comparing the oldest age group (80+ years) to the youngest (20–29 years), the values were 48% lower for both men and women. Across all age categories, women's absolute and relative $\dot{V}O_{2peak}$ values were, on average, lower when compared with men's values by −1.00 L/min (35%), −7.1 mL/kg/min (19%), and −4.7 mL/kg lean mass/min (10%).

For both men and women, quantile curves are provided for absolute $\dot{V}O_{2peak}$ (L/min), relative $\dot{V}O_{2peak}$ (mL/kg/min), and relative $\dot{V}O_{2peak}$ to lean body mass (mL/kg lean mass/min) in Figures 2a and 2b, respectively. Quantile curves of other relevant CPET parameters can be found in the supplemental digital content (SDC 1).

The mediation analysis revealed that the direct effect of age on $\dot{V}O_{2peak}$ was a predicted mean change of -0.28 ml/kg/min (95%-CI: -0.32; -0.26) for every additional year ($p < 0.001$) and the total indirect effect of age mediated over LPA, MPA and VPA was only very small with -0.06 ml/kg/min (95%-CI: -0.08; -0.04; $p < 0.001$). The total effect of age on $\dot{V}O_{2peak}$ was a predicted mean change of -0.35 ml/kg/min (95%-CI: -0.38; -0.32) for every additional year ($p < 0.001$).

Table 2. *Empirical CPET data of the study population separated by sex and decades. Data are presented as mean ± standard deviation and 15th;85th percentile*

	20-29		30-39		40-49		50-59		60-69		70-79		80+	
	male	female	male	female	male	female	male	female	male	female	male	female	male	female
Participants, no. (%)	37 (7.4)	30 (6.0)	40 (8.0)	33 (6.6)	36 (7.2)	35 (7.0)	35 (7.0)	35 (7.0)	40 (8.0)	36 (7.2)	41 (8.2)	38 (7.6)	35 (7.0)	31 (6.2)
Maximal parameters														
Peak $\dot{V}O_2$ (L/min)	3.54±0.58	2.35±0.3	3.41±0.55	2.41±0.34	3.36±0.7	2.26±0.29	3.08±0.62	2±0.31	2.8±0.45	1.59±0.27	2.27±0.42	1.49±0.25	1.85±0.28	1.2±0.23
	2.9;4.08	2.06;2.59	2.82;3.89	2.11;2.82	2.48;4.11	1.96;2.54	2.46;3.7	1.66;2.39	2.26;3.27	1.31;1.87	1.82;2.62	1.26;1.74	1.51;2.11	0.95;1.35
Peak $\dot{V}O_2$ (mL/kg/min)	46.6±7.9	39.3±6.5	44.7±7.4	38.9±5.8	44±9.2	34.7±4.4	38.4±8.5	31.9±4.7	37±6.9	26.3±5.4	30.6±5.9	24.4±3.9	24.5±4.3	20.6±3.6
	40;54.4	31;46.3	37.8;50.2	34.3;45.5	34.4;52.5	30.2;38.8	30;45	26.8;36.6	29.4;45	21.1;31.7	23.3;36.7	19.9;28.9	19.5;28.4	16.9;24.1
Peak $\dot{V}O_2$ (mL/kg leanmass/min)	54.7±7.5	51.6±6.7	53.1±7.5	51.1±6.6	52.3±9	45.8±4.2	48.1±8.8	43.9±5.9	46±6.9	37.8±6	40.3±6.8	34.8±4.9	33.5±5	30.5±5.8
	47.4;61.1	45.7;57.7	45.6;60.5	45;57	43;61.1	42.3;49.1	39.7;56.3	37.6;50	38.8;53.1	32.1;44.5	32.3;45.7	30.8;39.9	27.6;38.4	24.4;36.1
Peak O_2pulse (mL/beat)	17.5±2.2	12.6±1.7	17.3±2.3	13.3±2.2	17.4±2.5	13.4±2.2	16.8±2.5	11.8±1.9	16.7±2.1	10±1.8	14.7±2.3	10±1.6	13.6±2	8.9±1.4
	14.7;19.4	11.2;14.9	14.7;19.4	11.3;16.1	14.3;19.8	10.8;15.1	14.1;19.4	9.8;14.3	13.7;18.6	8.1;11.9	12.6;16.5	8.2;11.6	11.1;15.8	7.4;10.3
Peak $\dot{V}_E$ (l/min)	154±29	100±15	144±26	103±19	144±29	98±15	137±30	87±15	124±21	73±17	104±25	63±13	83±20	54±12
	121;186	85;109	123;169	80;121	110;173	84;114	103;178	68;103	98;144	56;87	75;125	50;77	65;102	41;67
Peak workload (W)	305±62	199±32	297±60	203±32	300±68	196±30	268±65	166±28	242±44	123±32	179±39	101±23	135±31	73±16
	252;360	165;229	252;355	172;236	230;367	172;232	201;347	134;196	194;288	91;156	143;227	78;124	101;166	54;91
Peak HR (bpm)	192±10	188±7	189±7	184±10	180±10	176±13	175±13	173±10	166±16	162±14	157±15	152±14	141±20	140±17
	182;199	182;194	182;194	175;192	174;189	167;190	161;189	163;183	146;183	144;175	145;172	136;163	120;162	122;154
Peak RER	1.22±0.06	1.21±0.06	1.22±0.06	1.2±0.04	1.2±0.06	1.21±0.07	1.2±0.06	1.21±0.05	1.18±0.07	1.17±0.06	1.13±0.08	1.12±0.09	1.09±0.08	1.08±0.07
	1.16;1.27	1.15;1.28	1.16;1.27	1.15;1.26	1.14;1.25	1.14;1.26	1.14;1.28	1.17;1.25	1.1;1.25	1.1;1.24	1.04;1.21	1.04;1.19	1.01;1.18	1.02;1.14
Peak Lac (mmol/L)	11.4±2	9.6±2.4	10.7±2	9.1±1.9	10±2.1	7.7±1.6	9±2.5	7.5±1.2	8±2.1	6.2±1.7	6.6±2.1	5.1±1.6	5±1.4	4.1±1.4
	9.1;13.5	7;12.4	8.4;12.8	7;11.1	7.9;12.1	6.2;9	7.2;11.3	6.4;8.9	5.9;10.1	4.6;8.2	4.6;8.6	3.5;7.2	3.4;6.2	2.6;5.4
Submaximal parameters														
$\dot{V}O_2$ at VT1 (mL/kg/min)	26.8±6.7	23.5±5.1	25.1±6	22.8±4.9	25.6±6.8	20.3±3.6	22.1±6	19.1±3.8	22.2±5.8	17±3.9	18.8±4.9	16.2±2.8	16.1±2.8	14.9±3.3
	21;32.8	18.4;29	18.9;29.2	18.6;27	19.3;33.8	16;24.9	16.1;29	16;23	15.9;28.2	13;21	14;23	14;18	13;19	12;19
$\dot{V}O_2$ at VT1 (L/min)	2.03±0.47	1.41±0.28	1.91±0.45	1.43±0.24	1.96±0.5	1.33±0.26	1.78±0.48	1.2±0.25	1.68±0.43	1.02±0.2	1.41±0.32	1±0.19	1.22±0.21	0.86±0.19
	1.63;2.58	1.11;1.73	1.54;2.3	1.19;1.67	1.47;2.51	1.05;1.57	1.3;2.21	0.94;1.43	1.19;2.1	0.84;1.24	1.12;1.64	0.84;1.14	0.99;1.44	0.64;1.07
$P_{ET}CO_2$ at rest (mmHg)	34.2±2.6	31.3±2.1	34±2.6	30.7±1.9	33.4±2.3	30.5±2.4	32.5±3.1	32.1±2.2	31.7±2.7	30.4±2	29.8±3.1	30.8±2.9	28.9±2.6	29.3±2.9
	32.4;36.6	28.6;33.1	31.5;36.3	28.7;32.7	31;36	28;33	30;36	30.1;34.5	28.4;34.2	28.3;32.5	27.3;32.7	27.8;33.4	26.2;31.5	26.6;32.2
$P_{ET}CO_2$ at VT1 (mmHg)	45.1±3.3	43±4.2	45.3±3.8	41.5±2.9	43.6±3.4	40.3±2.8	42.7±3	42±3.7	40.5±2.8	38.6±3.1	37.9±3.5	39.5±4.1	36±2.9	35.5±3.7
	42;48	37.4;46.7	42;48	38;44.2	40;47.8	38;42.9	41;45	38;45.9	37;43	36;41.8	35;42	35.6;43	33;39	32.5;38
$\dot{V}_E$/ $\dot{V}CO_2$ slope	35.3±5.3	33.7±6.3	34.2±5.8	34±5.6	35.5±7.2	34.3±4.4	36.9±6.1	35.8±6.6	37.2±4.7	38.6±7.5	39.6±8.7	36.4±5.8	38.6±5.9	40.7±5.4
	28.7;39.5	27.3;39.3	27.6;40	29.2;40.1	28.8;41	30.7;38.6	31.2;41.3	30.8;42.6	31.7;41.7	29.8;46.9	33.7;46.7	30.5;42	33.5;45	34.7;45
$\dot{V}_E$/ $\dot{V}CO_2$ slope below VT2	26.7±3.8	26.6±4.9	25.8±3.5	27.2±3.4	27.9±4.5	27.8±3.7	28.1±4	28.3±4.1	30.5±4.1	31.8±5.6	33±5.1	31.7±4.5	34.5±4.8	35.9±4.3
	22.8;30.5	21.3;31.1	22.5;28.8	23.2;30.2	24.7;31.4	24.4;31.2	24.5;31.2	24.6;32.6	26.6;35.6	26.4;38.9	28.2;39.2	26.1;36.3	29.2;39.5	30.8;39
OUES* (mL/min)	3426±633	2478±540	3391±637	2549±385	3417±867	2384±402	3068±599	2113±446	2939±581	1703±325	2452±544	1680±327	2180±439	1416±284
	2737;4061	1999;3159	2495;3915	2135;2878	2435;4277	1912;2807	2548;3571	1643;2602	2236;3474	1433;1971	2027;3090	1374;2009	1809;2505	1108;1719
OUES* (mL/min/kg)	45.1±8.7	41.3±9.3	44.5±8.7	40.7±8.4	44.6±11.6	36.8±6.8	38.4±8.8	33.8±6.9	38.8±8.7	28.6±7	32.6±8	27.5±5.3	29±5.9	23.9±5.5
	37.4;53.2	31.5;50.8	36.3;53.1	32.8;48	33;57.9	30.2;45.2	30.8;47.8	28.2;41	30.6;47.7	22.3;35.3	25.4;39.8	21.2;33.3	23.3;34.5	19.9;29

Abbreviations: $\dot{V}O_2$, oxygen uptake; $\dot{V}E$, volume of expiration; HR, heart rate; RER, respiratory exchange ratio; VT, ventilatory threshold; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; $\dot{V}CO_2$, carbon dioxide output; OUES, oxygen uptake efficiency slope; HRR, heart rate recovery.

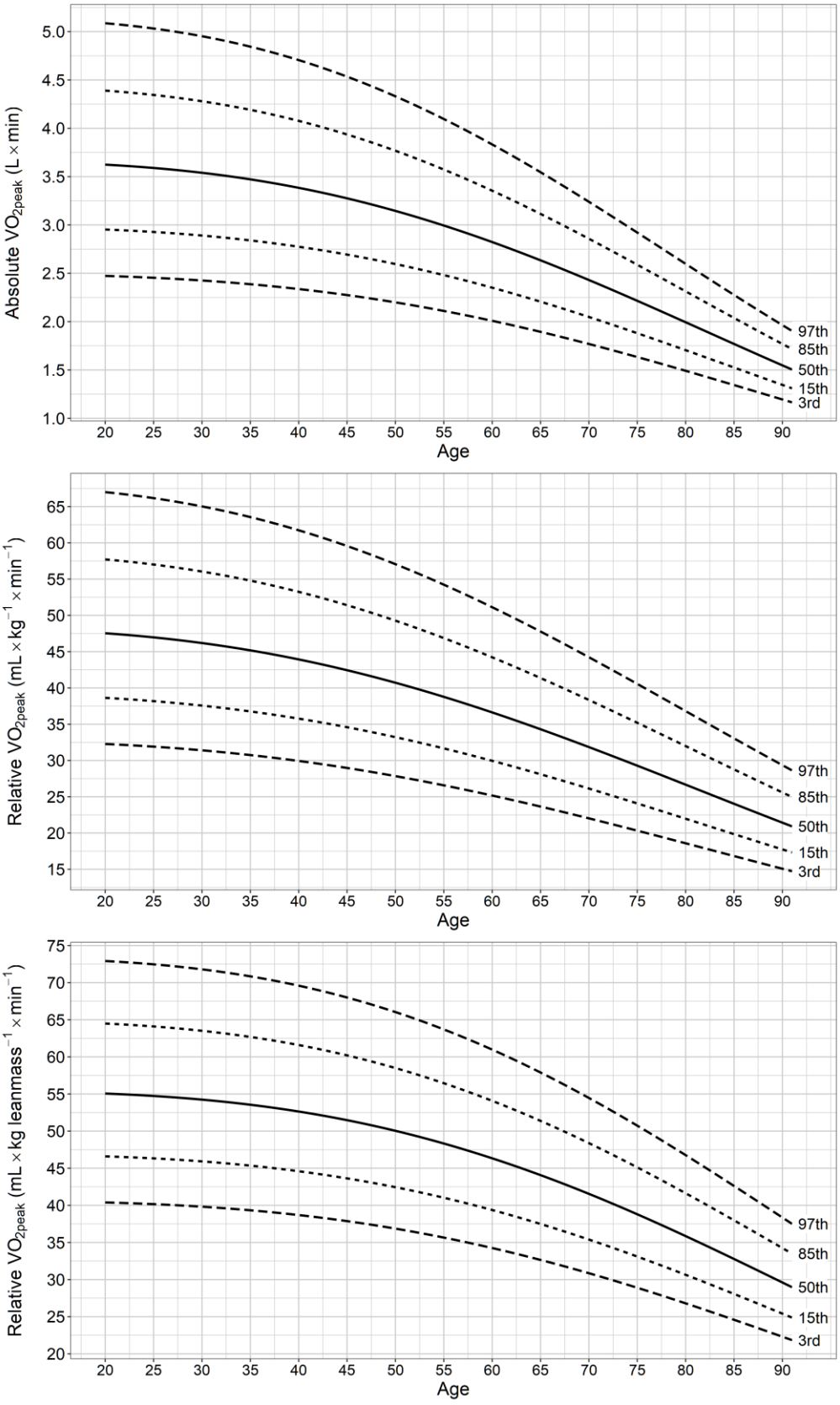

***Figure 2a**: Quantile curves for men for absolute $\dot{V}O_{2peak}$ (L/min), relative $\dot{V}O_{2peak}$ (mL/kg/min), and relative $\dot{V}O_{2peak}$ to lean body mass (mL/kg lean mass/min).*

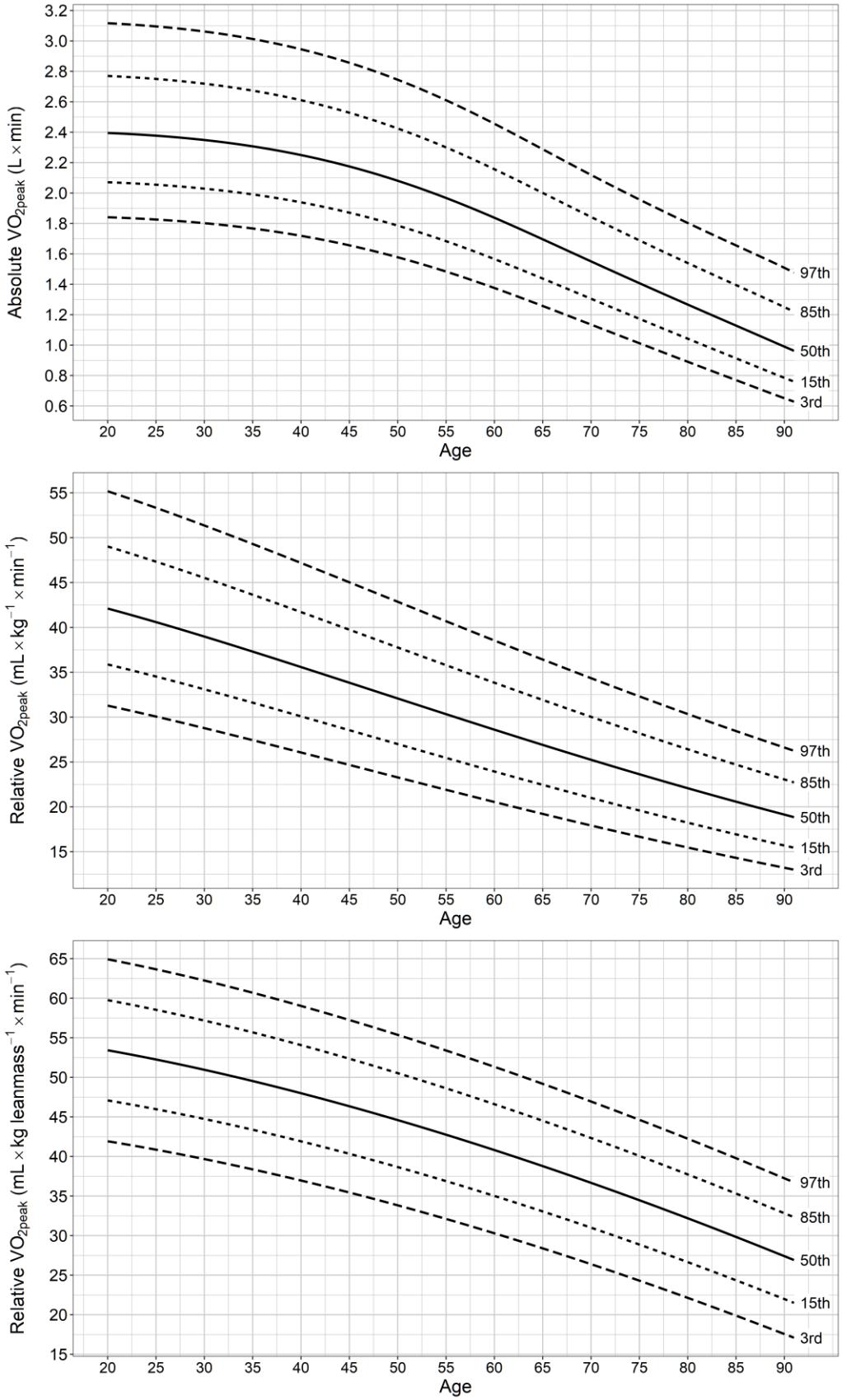

***Figure 2b**: Quantile curves for women for absolute $\dot{V}O_{2peak}$ (L/min), relative $\dot{V}O_{2peak}$ (mL/kg/min) and relative $\dot{V}O_{2peak}$ to lean body mass (mL/kg lean mass/min).*

Comparison with other reference values

Relative and absolute $\dot{V}O_{2peak}$ values from all major previous studies reporting CPET reference values for tests performed on cycle ergometers (6, 7, 13, 26-28) are presented for men and women separately in Figures 3a and 3b. The absolute and relative $\dot{V}O_{2peak}$ values of previous studies were, on average, 0.34 (L/min) (12%) and 7.5 (mL/kg/min) (20%) lower relative to those for males and 0.24 (L/min) (13%) and 6.5 (mL/kg/min) (21%) lower relative to those for females in our COmPLETE-Health Study, respectively. These differences ranged from -0.58 (L/min) (-19%) to +0.34 (L/min) (+8%) and -11.8 (mL/kg/min) (30%) to -0.39 (-1%) for males and -0.51 (L/min) (25%) to +0.2 (+3%) and -12.1 (mL/kg/min) (-38%) to -0.28 (mL/kg/min) (-7%) for females when comparing previous studies with the present study.

Association of PA and CPET parameters

Association of key CPET parameters and light, moderate and vigorous PA are presented in Table 3 and are graphically illustrated in Figure 4 and in SDC 2. There was strong evidence supporting the existence of positive associations between $\dot{V}O_{2peak}$ and the levels of habitual PA performed at MPA and VPA (Figure 4). However, there was weak evidence for an association between $\dot{V}O_{2peak}$ and LPA. Further, there was little evidence for associations between LPA and any of the other tested CPET parameters expect for the OUES which presented evidence for a negative association. Further, there was evidence for positive associations between MPA and $\dot{V}O_2$ at VT1, peak oxygen pulse and OUES. Finally, and in accordance to MPA, there was strong evidence for associations between VPA and $\dot{V}O_2$ at VT1, peak oxygen pulse and OUES. Only little evidence was observed for an existence of an association between VPA and $\dot{V}E/\dot{V}CO_2$ slope or $PETCO_2$ at VT1.

Table 3. *Association of Physical Activity and CPET parameters*

	Light Physical Activity		**Moderate Physical Activity**		**Vigorous Physical Activity**	
Parameter	**estimate (95%-CI)**	***p*-value**	**estimate (95%-CI)**	***p*-value**	**estimate (95%-CI)**	***p*-value**
$\dot{V}O_{2peak}$ (mL/kg/min)	−0.012 (−0.033; 0.010)	0.30	0.020 (0.007; 0.033)	0.003	0.260 (0.194; 0.325)	<0.001
$\dot{V}O_2$ at VT1 (mL/kg/min)	−0.005 (-0.023; 0.014)	0.63	0.017 (0.006; 0.027)	0.002	0.196 (0.136; 0.256)	<0.001
Peak oxygen pulse (mL/beat)	−0.004 (−0.012; 0.004)	0.32	0.006 (0.001; 0.010)	0.01	0.046 (0.021; 0.070)	<0.001
OUES (mL/min)	-2.147 (−4.027; −0.267)	0.03	1.314 (0.247; 2.380)	0.02	12.640 (5.631; 19.590)	<0.001
$\dot{V}E/\dot{V}CO_2$ slope	0.004 (−0.014; 0.021)	0.68	−0.008 (−0.018; 0.002)	0.13	−0.014 (−0.064; 0.036)	0.59
$P_{ET}CO_2$ at VT1 (mmHg)	−0.004 (−0.018; 0.010)	0.61	0.005 (−0.003; 0.012)	0.21	0.015 (−0.022; 0.052)	0.42

Abbreviations: 95%-CI, 95% confidence interval; $\dot{V}O_2$, oxygen uptake; $\dot{V}E$, volume of expiration; VT, ventilatory threshold; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; $\dot{V}CO_2$, carbon dioxide output; OUES, oxygen uptake efficiency slope.

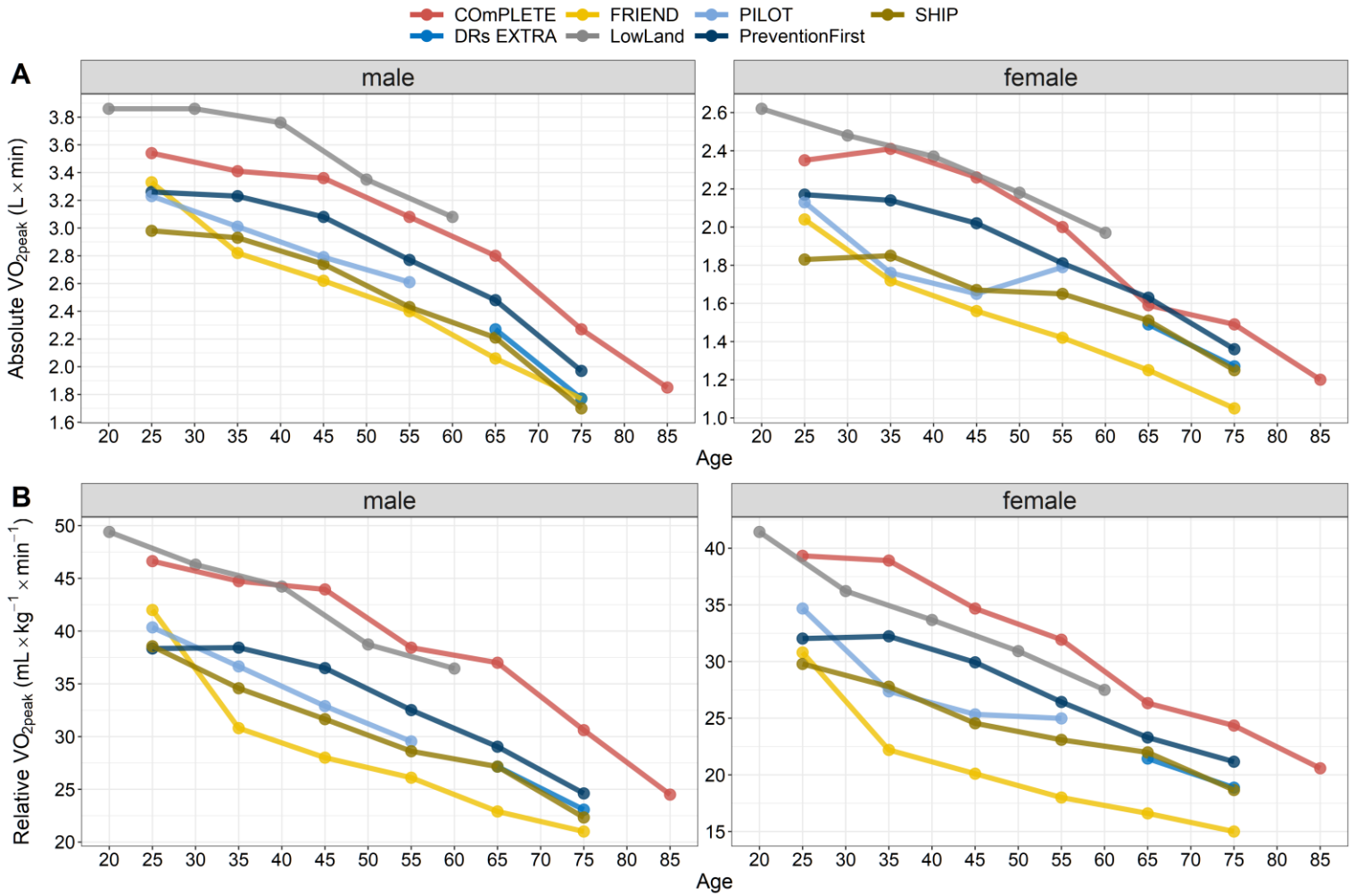

Figure 3: *Comparison of relative and absolute V̇O2peak reference data sets of all major studies reporting CPET reference values for tests performed on cycle ergometers*

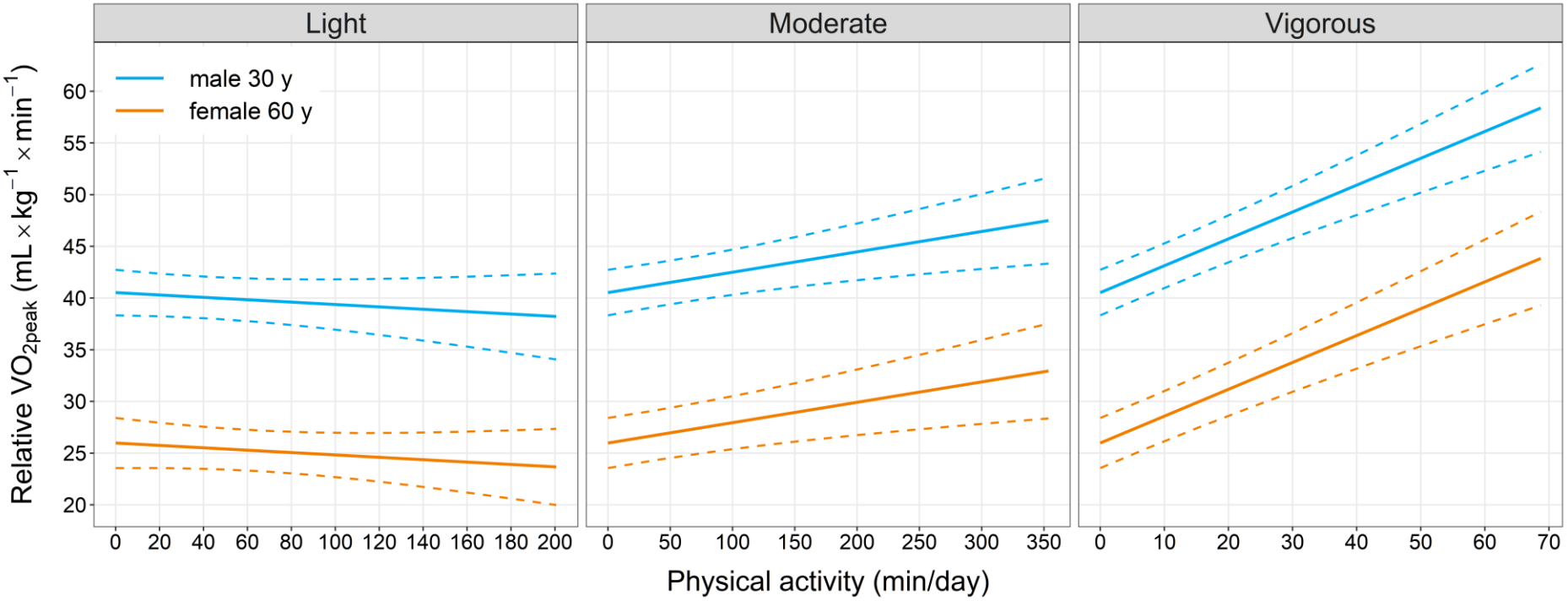

Figure 4: *Associations of LPA, MPA, and VPA and relative V̇O2peak.*

Legend Figure 4: To visualize the associations between relative V̇O2peak and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Discussion

CPET parameters and comparison with other reference values

This study presents quantile curves and empirical data for several maximal and submaximal CPET parameters in a prospectively assessed population-based healthy sample consisting of 502 subjects aged from 20 to 90 years. Our data represent the first (5) reference dataset meeting 12 out of 14 CPET standards, according to the 2003 American Thoracic Society and American College of Chest Physicians statement on exercise tests (11) (SDC 3). When comparing the current dataset with all other published datasets established with CPET performed on cycle ergometers, the COmPLETE-Health Study clearly stands out with high $\dot{V}O_{2peak}$ values, only the Low Land Fitness Registry shows similar data (13). Remarkable results include the large differences seen between these studies' reference values, which have potential practical and clinically relevant consequences when applying one or the other reference dataset. For example, a 60-year-old woman with a $\dot{V}O_{2peak}$ of 19 mL/kg/min would be classified in the 80th percentile (top 20%) according to the FRIEND registry (7), but would be below the third percentile in our COmPLETE-Health Study. Ranking below the third percentile is a clinical indication to search for possible underlying pathologies for the reduced cardiorespiratory fitness (CRF). When looking at the percentile table of the FRIEND registry (7), it seems that a substantial proportion of the individuals in higher age groups (60 years and up), especially women, had $\dot{V}O_{2peak}$ values less than 14 mL/kg/min, and thus would be potential candidates for heart transplantation (29). It should be, however, noted that the criteria for heart transplantation most likely refers to tests performed on treadmills even though it is not stated in the guidelines. $\dot{V}O_{2peak}$ values observed on cycle ergometers are usually 2- 12% lower as compared to tests performed on treadmills (30-32). Furthermore, the heart transplantation criterion recommended by the International Society for Heart Lung Transplantation (29) for younger patients (< 50 years) is a $\dot{V}O_{2peak}$ below 50% of its age-related value, but the guidelines do not indicate which reference values should be used for this evaluation.

Reasons explaining the low values of the studies shown in Figure 3 could be, amongst others, the non–population-based approach of several reference studies using CPET data sampled in hospitals rather than recruiting a healthy reference population. Participants in hospitals are prone to have a clinical indication to perform CPET (e.g., shortness of breath). Even though the included participants of these studies have not been diagnosed with cardiovascular disease, they probably presented with concerns regarding physical performance or exercise tolerance. Further, differences in exclusion criteria vary widely. The present study excluded all types of manifest exercise limiting chronic disease, whereas most studies just exclude cardiovascular disease but not cancer, diabetes, or other circumstances affecting the CPET parameters either directly or indirectly through reduced PA. Also, many studies include participants having risk factors such as smoking or obesity. To note, a large proportion of the studied sample in the FRIEND registry (7) between the 30 to 70 years was obese, with an average BMI of 29 kg/m^2 or greater which at least

partly explains the low relative $\dot{V}O_{2peak}$ values. In addition, PA patterns between these studies and countries are most likely to differ, with European studies typically showing higher $\dot{V}O_{2peak}$ values than American studies. In particular, the present (Swiss) and a previous Dutch study (33) showed the highest $\dot{V}O_{2peak}$ values, with both countries known for high PA levels in the general population (34). To investigate potential selection bias, we compared the habitual PA levels of our cohort to those of the COLAUS cohort, the largest Swiss cohort study (with the same accelerometer used) (35). The medians and interquartile ranges for minutes performed at LPA, MPA, and VPA per day were nearly identical at 96 (78–117), 160 (119–199) and three (1–9) minutes for our study and 106 (84–130), 162 (199–220), and two (0–5) minutes for the COLAUS cohort, respectively. Finally, several reference values studies have applied no (6, 26) or relatively low secondary exhaustion criteria (13, 26, 28, 36, 37) such as RER of ≥1.0 or ≥85% of age-predicted maximal heart rate. We have recently shown that, when using a low exhaustion criterion such as a RER of 1.0 participants reached on average only 72% ± 14% of their individual $\dot{V}O_{2peak}$ (18, 38). It can therefore be postulated that studies not or insufficiently applying exhaustion criteria substantially underestimate their participants' aerobic fitness levels. Exhaustion criteria urgently need to be applied, especially as not all studies have established standardized instructions for the investigators to push the participants to their individual physiological limit.

Beside the differences in the reported results by this study and the results provided by FRIEND registry and other studies described above, it should be noted, that they show several similar observations. The percent reduction per decade of $\dot{V}O_{2peak}$ (Figure 3) is comparable between the studies analyzed including the FRIEND registry and the present COmPLETE-Health Study with approximately 10% per decade. Further, it should be noted that large differences between reference values for CPETs performed on cycle ergometers were also observed by the FRIEND registry (7). The current manuscript, therefore, strengthens this observation and the need for CPET reference values from different geographical regions.

Clinicians and scientists need to be aware of these large differences in $\dot{V}O_{2peak}$ reference values. The comparison to other studies was systematically performed for $\dot{V}O_{2peak}$ but it can be expected that the other reported submaximal and maximal CPET parameters related to cardiorespiratory health and performance show similar results. The appropriate set of reference values should be selected on a case-by-case basis. When choosing a reference dataset, the following points should be considered: origin of the data (country/geographic region), recruitment of the studied sample (population-based or hospital-based), in- and exclusion criteria, and PA levels of the population (if reported). To distinguish a normal response from an abnormal (pathological) response to CPET, we recommend comparing participants/patients with a reference set composed of healthy subjects rather than with a dataset being representative of the average population. Especially in older age groups, the proportion of people living with a chronic disease and having risk factors, which affects aerobic capacity and CPET parameters directly or indirectly by reduced PA levels, impacts reference values.

Quantile curves of the submaximal parameters OUES, $\dot{V}E/\dot{V}CO_2$ slope and $P_{ET}CO_2$ were diversely shaped (SDC 1) but all trended toward pathological values with increasing age. To our knowledge, this is the first study reporting reference values of these parameters in a healthy population over an age range from 20-90 years. The OUES values for men and women between 20 and 60 were nearly identical between this study and a previous European study which is part of the Lowland registry (12). With the exception of females older than 80 years, all age groups easily met the proposed cutoff values of OUES for risk stratification with 1400 to 1470 (ml/min) (39, 40). A $\dot{V}E/\dot{V}CO_2$ slope of 34 is often proposed as a dichotomous cutoff to evaluate the prognosis of heart failure patients (41). Based on this cutoff, 17% of participants older than 60 years would have been classified as high-risk patients, despite being free from heart diseases. Therefore, the use of an age-corrected cutoff for $\dot{V}E/\dot{V}CO_2$ slope may be discussed. Furthermore, $\dot{V}E/\dot{V}CO_2$ slope values differ largely whether they were determined using all data or data up to the second ventilatory threshold.

The cutoff values for risk stratification of greater than 31 to 33 mmHg for resting $P_{ET}CO_2$ and greater than 36 mmHg (10) for $P_{ET}CO_2$ at VT1 were not reached by only a few subjects from 70 years and older. Overall, the application of dichotomous, age-independent cut-offs of CPET parameters in old and, in particular, very old subjects must be regarded as critical for risk stratification.

Association of PA and CPET parameters

To the authors' knowledge, this is the first population-based study that analyzed the relationship between objectively measured PA and directly measured cardiorespiratory fitness ($\dot{V}O_{2peak}$) in healthy men and women over such a wide age range. We observed that $\dot{V}O_{2peak}$ among men and women was higher when they performed more moderate- and vigorous-intensity PA. Minutes of PA performed at light, moderate, and vigorous intensities together with age and sex explain 67% of the variance in the participants' $\dot{V}O_{2peak}$ (mL/kg/min) values.

Especially, activities performed at vigorous intensity had a strong association with CRF, as shown in Figure 4. A clinically relevant difference in $\dot{V}O_{2peak}$ (an increase of ≥ 1 mL/kg/min) (42) was observed with as low as four minutes of additional VPA per day. Further, a 1-MET higher CRF (3.5 mL/kg/min) which corresponds to an approximately 15% lower incidence of myocardial infarction (43), was associated with 13 minutes of additional VPA per day. These two examples demonstrate the practical relevance of this finding and highlight the potential beneficial impact of higher-intensity exercise (≥7 METS) in improving CRF. In accordance with the population-based HUNT (44) study, which assessed subjective PA data, our results from objective PA data show that LPA, regardless of the number of minutes performed at said intensity, was not associated with higher levels of $\dot{V}O_{2peak}$. LPA in our study was defined as exercise of 1.5 to 3.9 METs, which seems to be too low to elicit changes in $\dot{V}O_{2peak}$ in healthy individuals. The intensity needed to elicit changes in $\dot{V}O_{2peak}$ seems, therefore, to be at least 4 METS in our healthy population. The concept of a minimum threshold of intensity to achieve changes in $\dot{V}O_{2peak}$ is supported by previous findings

(44-46), although the results may differ in a more sedentary population (46, 47). Our mediation analysis revealed that the role of LPA, MPA and VPA as mediators in the association between age and $\dot{V}O_{2peak}$ is very limited. The strong direct association between VPA levels and $\dot{V}O_{2peak}$ independently of age is, therefore, further strengthened.

Submaximal performance-related CPET parameters ($\dot{V}O_2$ at VT1 and OUES) showed similar associations with MPA and VPA as $\dot{V}O_{2peak}$. These results suggest that across the whole cohort, LPA was a too low stimulus to increase oxygen uptake at the submaximal level (VT1) or enhance oxygen uptake efficiency. As was to be expected, clinical CPET variables such as $P_{ET}CO_2$ and $\dot{V}E/\dot{V}CO_2$ slope did not show evidence for associations with PA levels, which may be explained by the healthy physiological levels of these parameters even in subjects of higher ages.

Limitations

Selection bias through our recruitment strategy, considering an inclusion rate of 3% to 5% of invitations sent, cannot be excluded and it is likely that our study participants had improved physical function and health status relative to those individuals who received an invitation but did not take part in the study. However, since we were not trying to recruit a representative sample of Swiss citizens but rather a sample of healthy male and female individuals across age groups, we think the potential selection bias is a negligible problem considering our aims. A negative correlation between PA and physical fitness and some exclusion criteria such as high blood pressure, a body mass index of 30 kg/m^2 or greater, or chronic disease can be assumed. The individuals meeting the inclusion criteria might, therefore, be more fit and active than comparable age group members not meeting the inclusion criteria. Further, the provided reference values may not be accurate for individuals with non-European origin. The cross-sectional design does not allow for conclusions to be made about causality for the presented associations. The associations observed between PA and CPET parameters could also be interpreted to indicate that the individuals who are capable and motivated to exercise more vigorously tend to be those who naturally have a higher CRF.

Conclusion

Submaximal and maximal CPET parameters assessed in a healthy population sample over such a large age range on a cycle ergometer are novel, of highest clinical relevance and offer a reference dataset for primary prevention and risk stratification. Reference values need to be chosen carefully to ensure a reliable interpretation of CPET results because differences in $\dot{V}O_{2peak}$ values between studies are distinct. Objectively measured vigorous-intensity PA has a strong association with higher $\dot{V}O_{2peak}$ and other performance-related CPET variables and should be measured as an important mediator of aerobic capacity.

List of abbreviations

CPET	cardiopulmonary exercise testing
CRF	cardiorespiratory fitness
LPA	light physical activity
MPA	moderate physical activity
OUES	oxygen uptake efficiency slope
PA	physical activity
$P_{ET}CO_2$	partial pressure of end-tidal CO_2
RER	respiratory exchange ratio
SEM	structural equation model
$\dot{V}E/\dot{V}CO_2$ slope	relationship of the ventilation to carbon dioxide production slope
$\dot{V}O_{2peak}$	peak oxygen uptake
VPA	vigorous physical activity
VT1	ventilatory threshold 1
VT2	ventilatory threshold 2

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451) and complied with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Consent for publication: not applicable

Availability of data and material: The datasets used for the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Author contributions:

Conceptualization, J.W., R.K., D.S., H.H., T.H., A.S.T.
Methodology, J.W., R.K., T.H., and A.S.T.
Formal analysis, D.I. and J.W.
Investigation, J.W., R.K. K.K., C.K., and J.C.
Resources, A.S.T.
Data curation, J.W. and R.K.
Writing—original draft, J.W.
Writing—review and editing, R.K., D.I., K.K., C.K., J.C., H.H., T.H., D.S., A.S.T.
Project administration, J.W. and R.K.

Funding acquisition, A.S.T.

All authors have read and approved the final manuscript.

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Supplemental digital content (sdc)

SDC 1: Quantile curves of additional relevant CPET parameters

SDC 2: Association of key CPET parameters and physical activity

SDC 3: Methodological quality list according to Takken et al. 2019 and the ATS/ACCP guidelines for CPET reference values

Table S1: Methodological quality list according to Takken et al. 2019 and the ATS/ACCP guidelines for CPET reference values

SDC 1: Quantile curves of additional relevant CPET parameters

Figure SDC 1.1: Quantile curves for men for Peak O_2pulse (mL/beat)

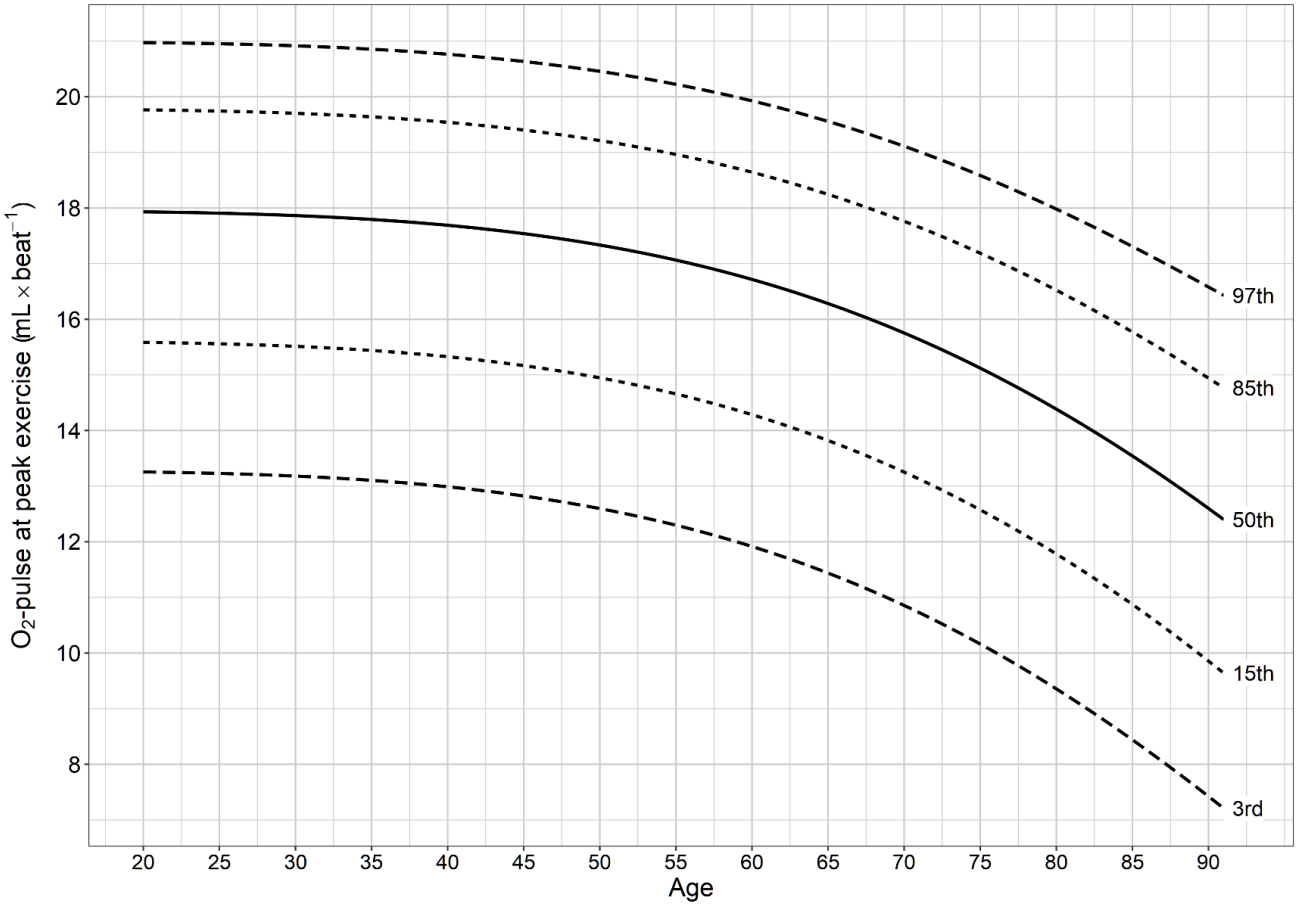


Figure SDC 1.2: Quantile curves for women for a Peak O_2pulse (mL/beat)

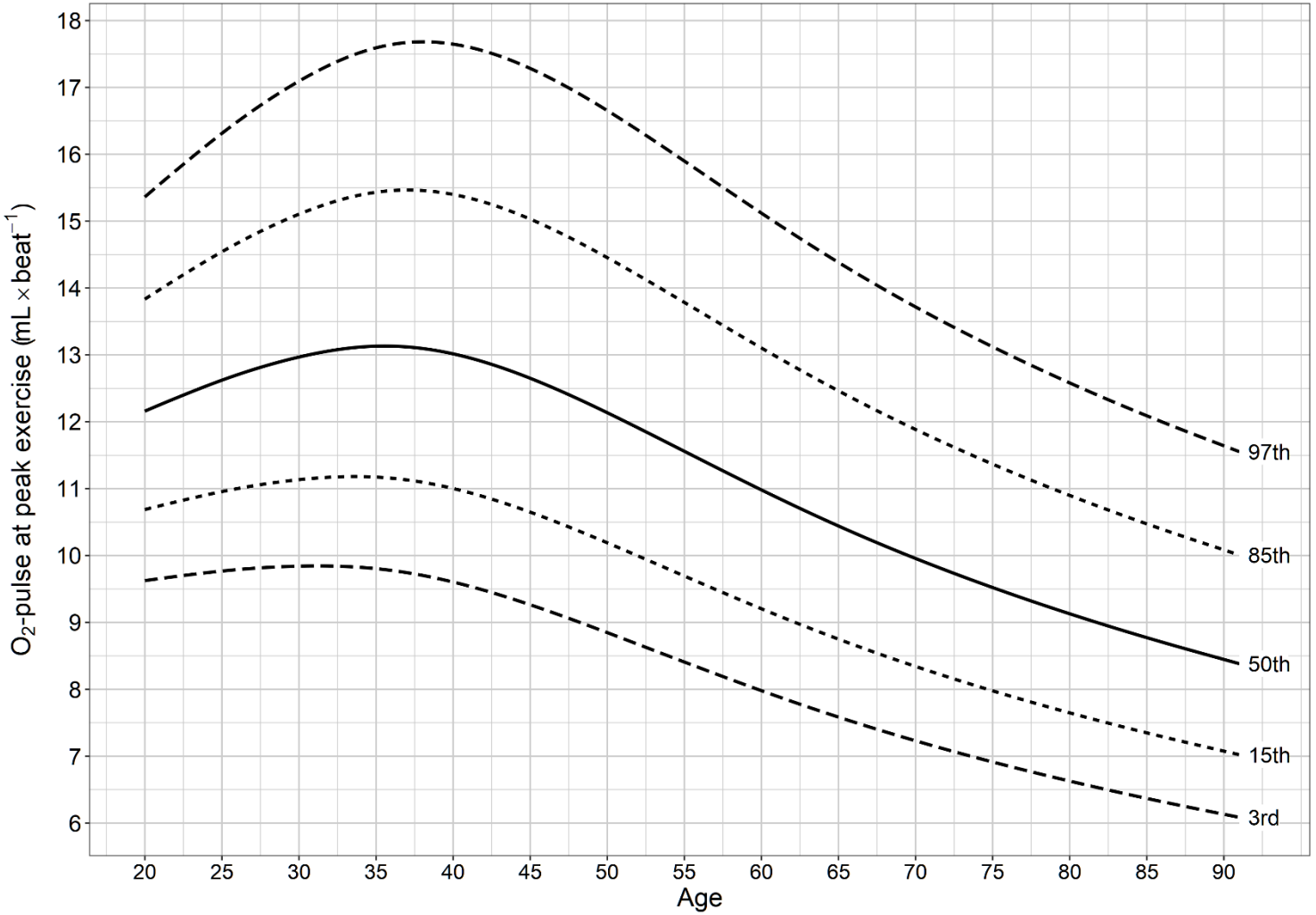


Figure SDC 1.3: Quantile curves for men for Peak workload (W)

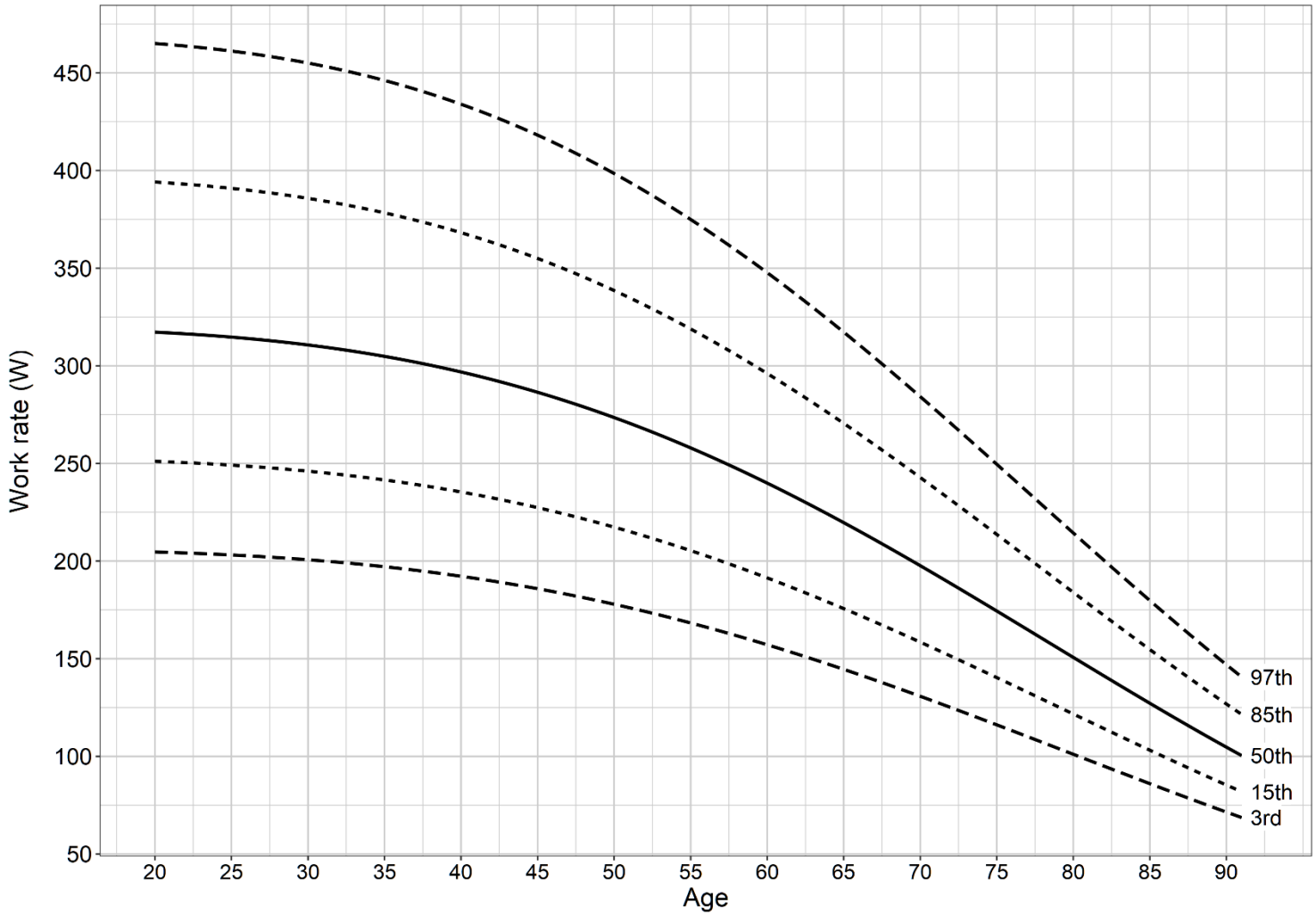


Figure SDC 1.4: Quantile curves for women for Peak workload (W)

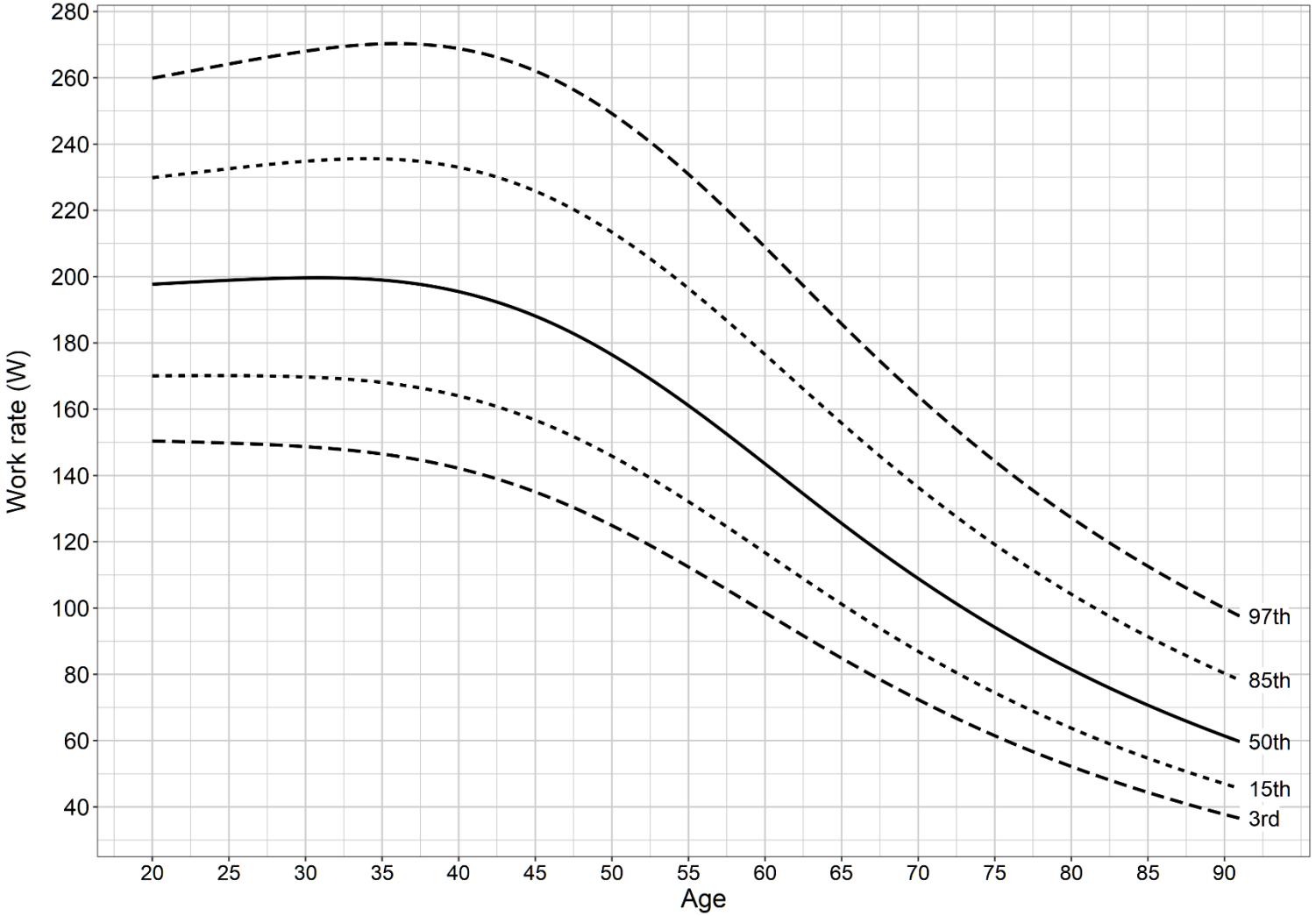
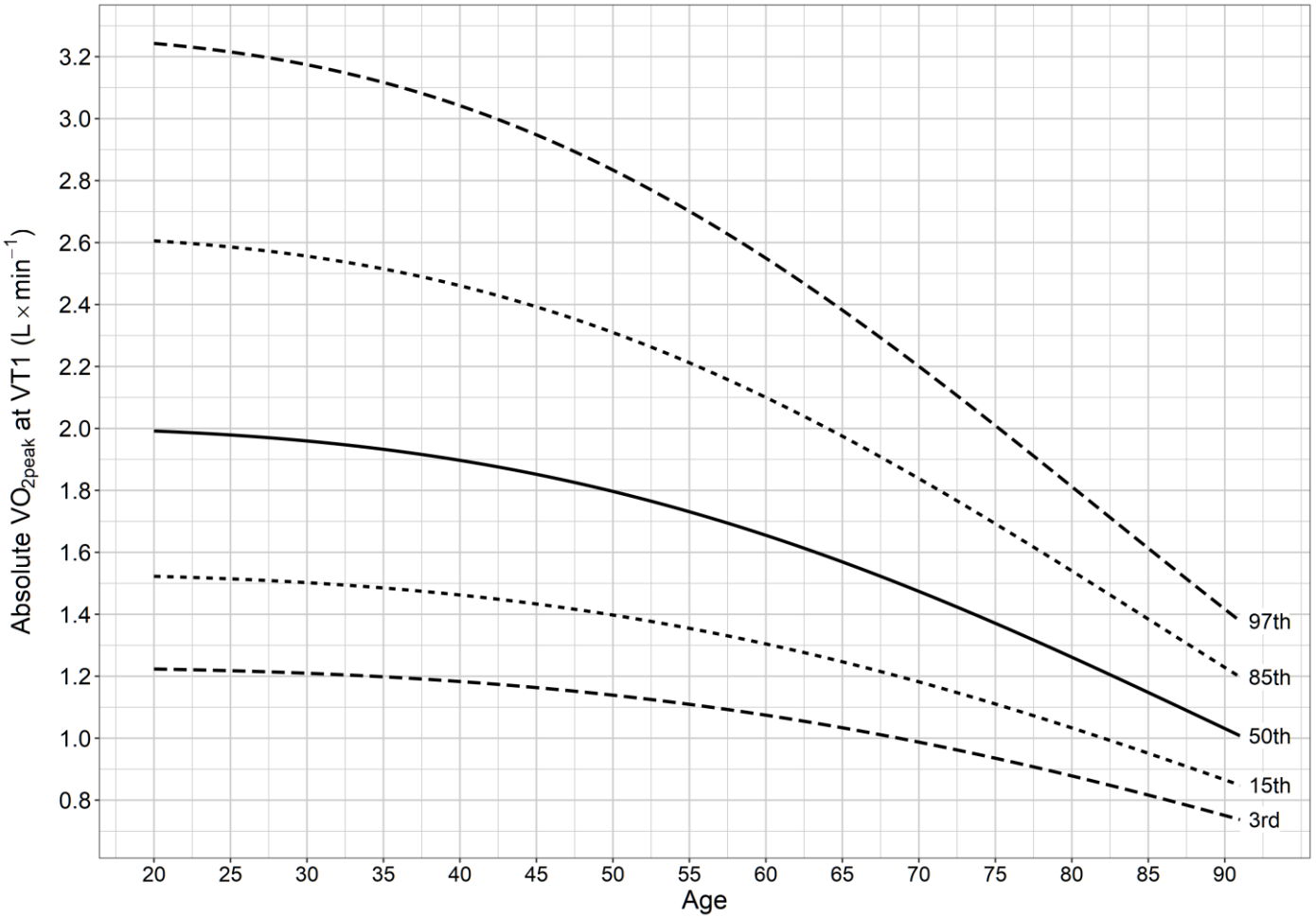
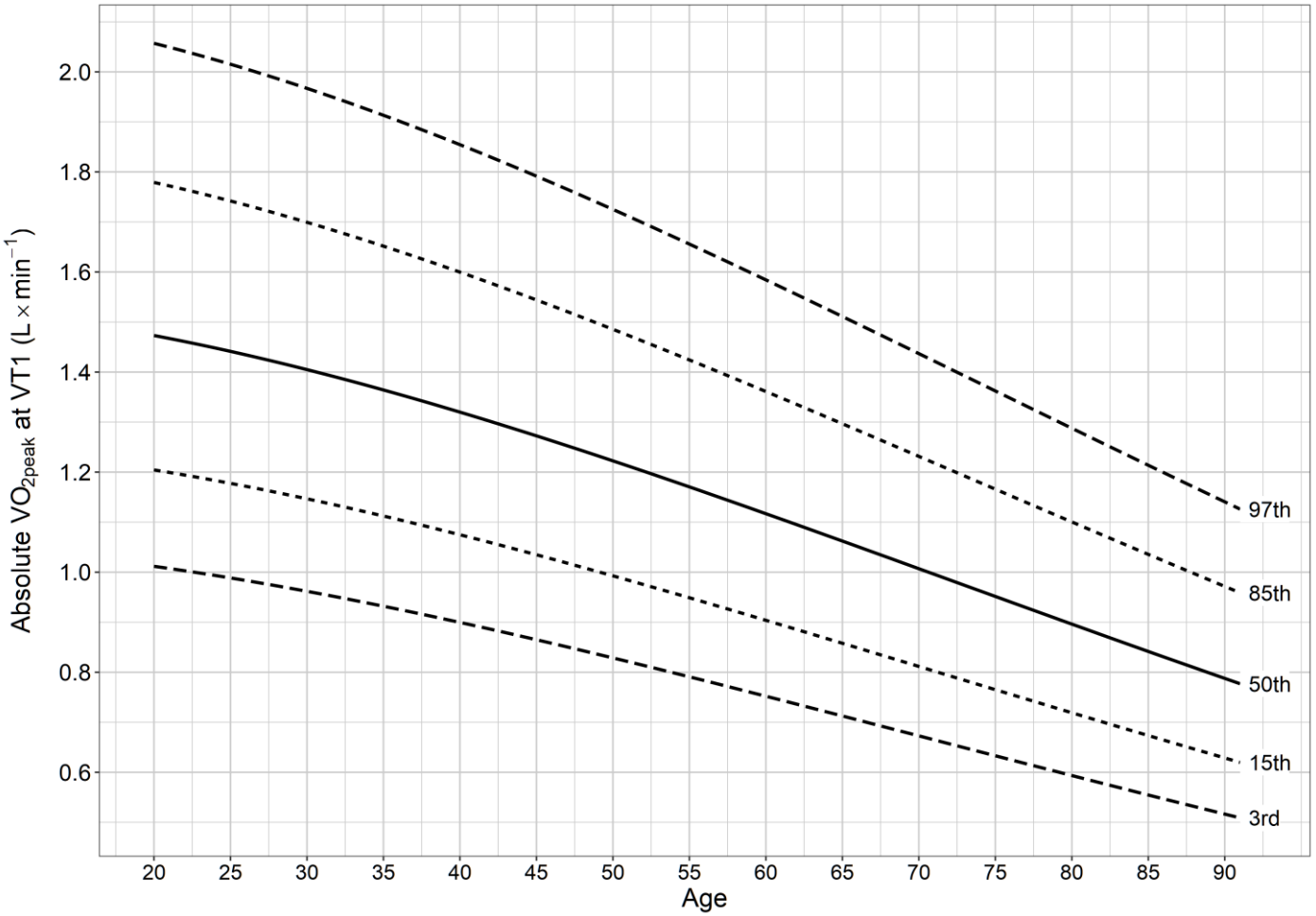


Figure SDC 1.5: Quantile curves for men for $\dot{V}O_2$ at VT1 (L/min)



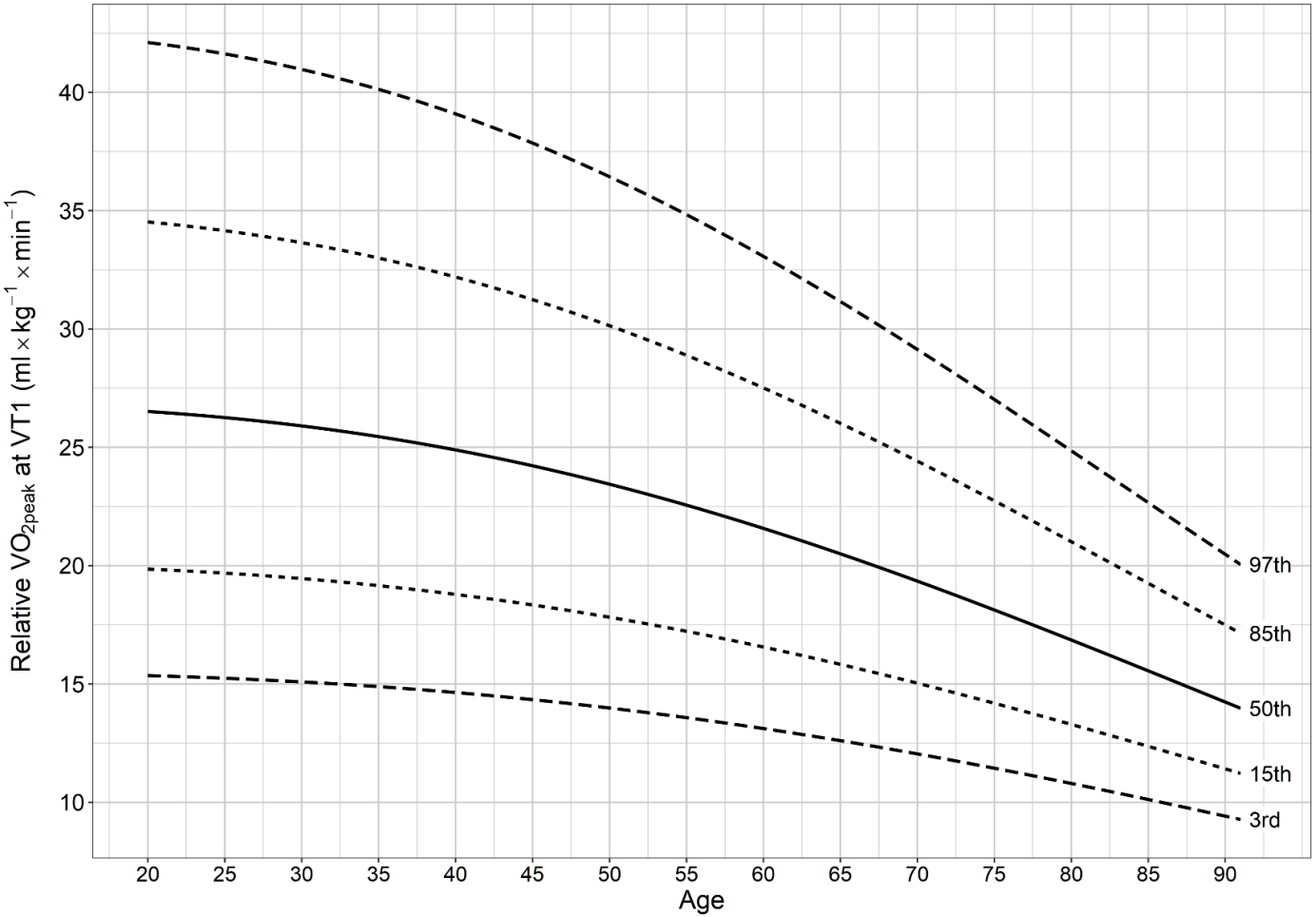
Abbreviations: $\dot{V}O_2$, oxygen uptake; VT1, ventilatory threshold 1

Figure SDC 1.6: Quantile curves for women for $\dot{V}O_2$ at VT1 (L/min)



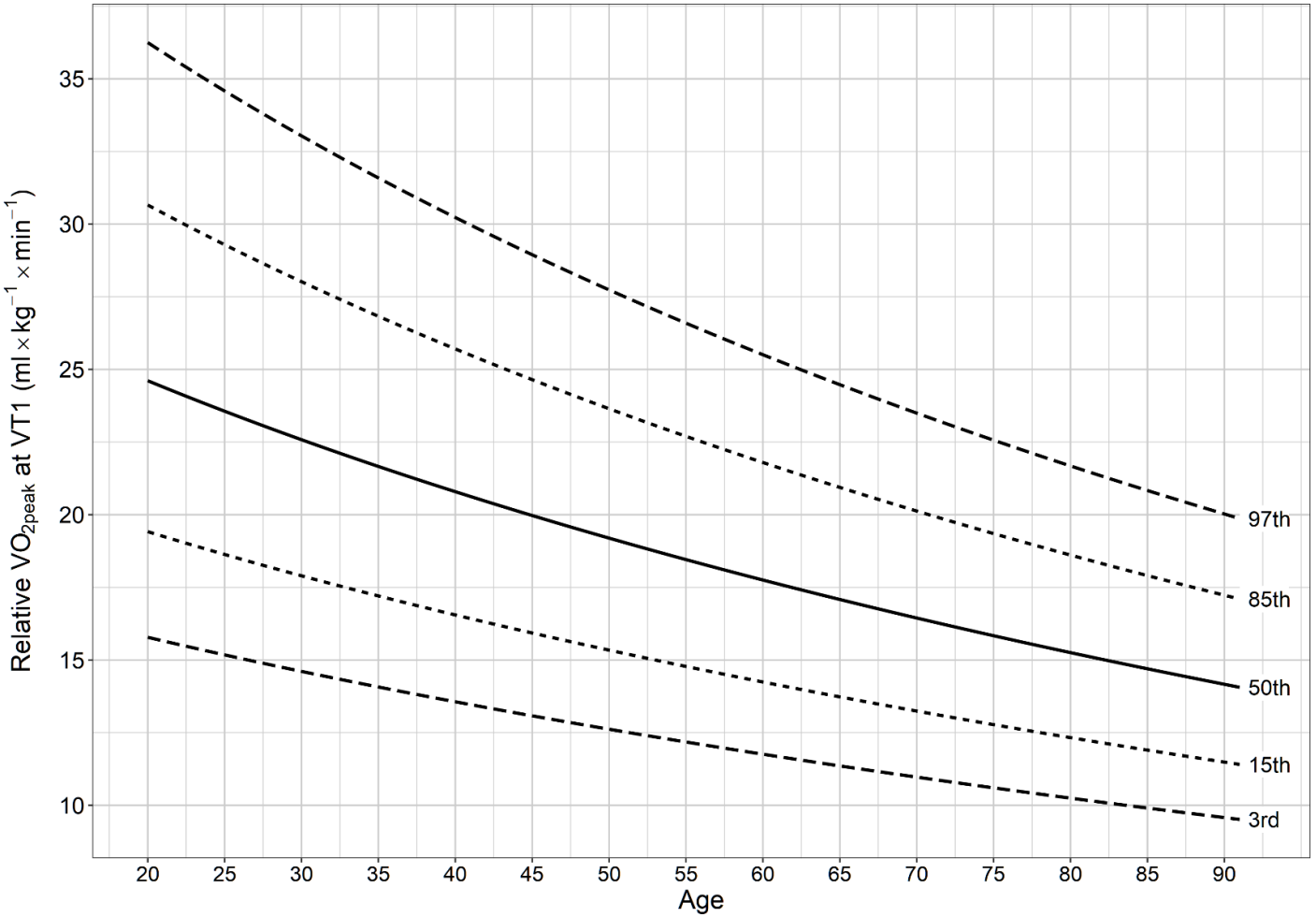
Abbreviations: $\dot{V}O_2$, oxygen uptake; VT1, ventilatory threshold 1

Figure SDC 1.7: Quantile curves for men for $\dot{V}O_2$ at VT1 (mL/kg/min)



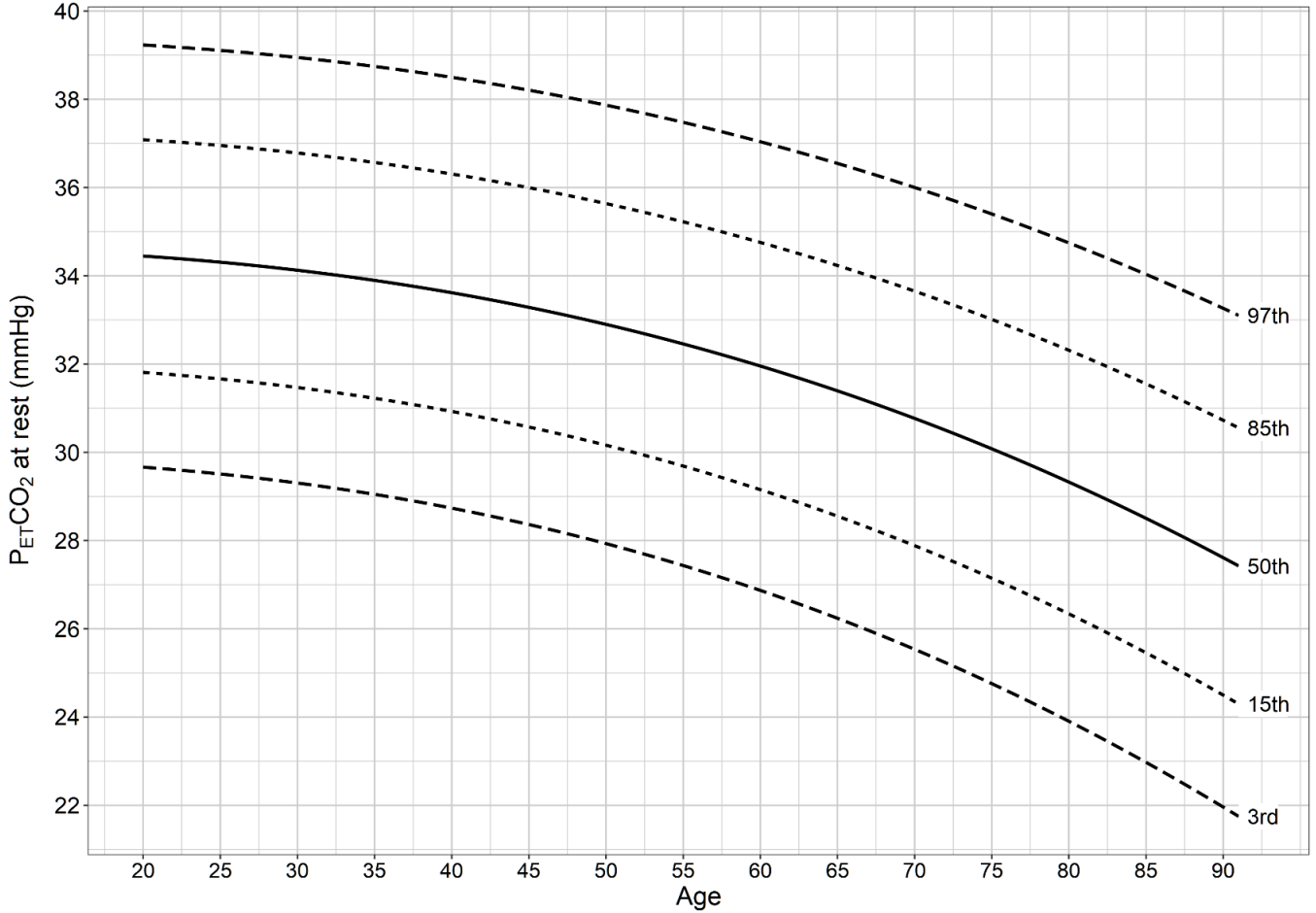
Abbreviations: $\dot{V}O_2$, oxygen uptake; VT1, ventilatory threshold 1

Figure SDC 1.8: Quantile curves for women for $\dot{V}O_2$ at VT1 (mL/kg/min))



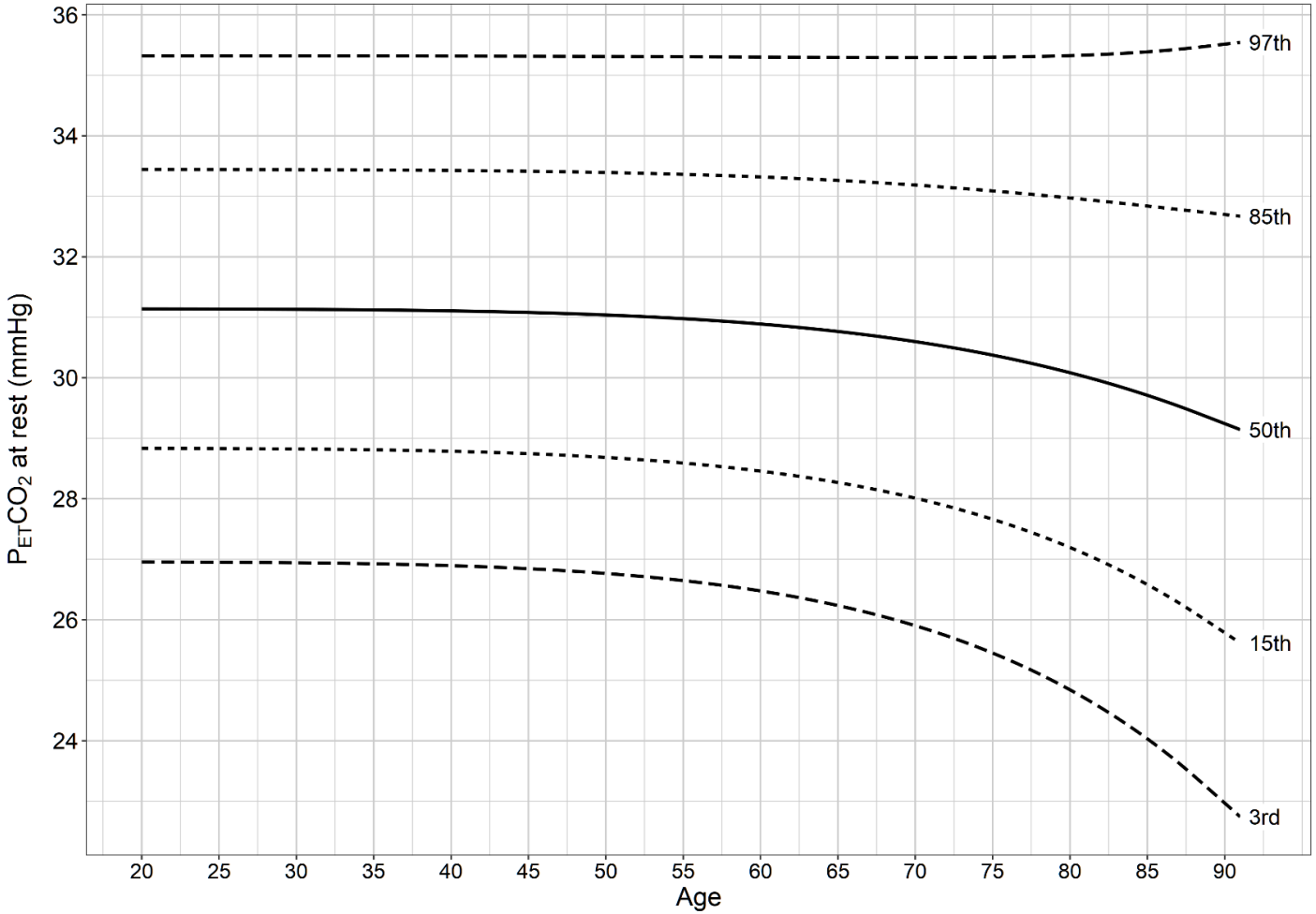
Abbreviations: $\dot{V}O_2$, oxygen uptake; VT1, ventilatory threshold 1

Figure SDC 1.9: Quantile curves for men for $P_{ET}CO_2$ at rest (mmHg)



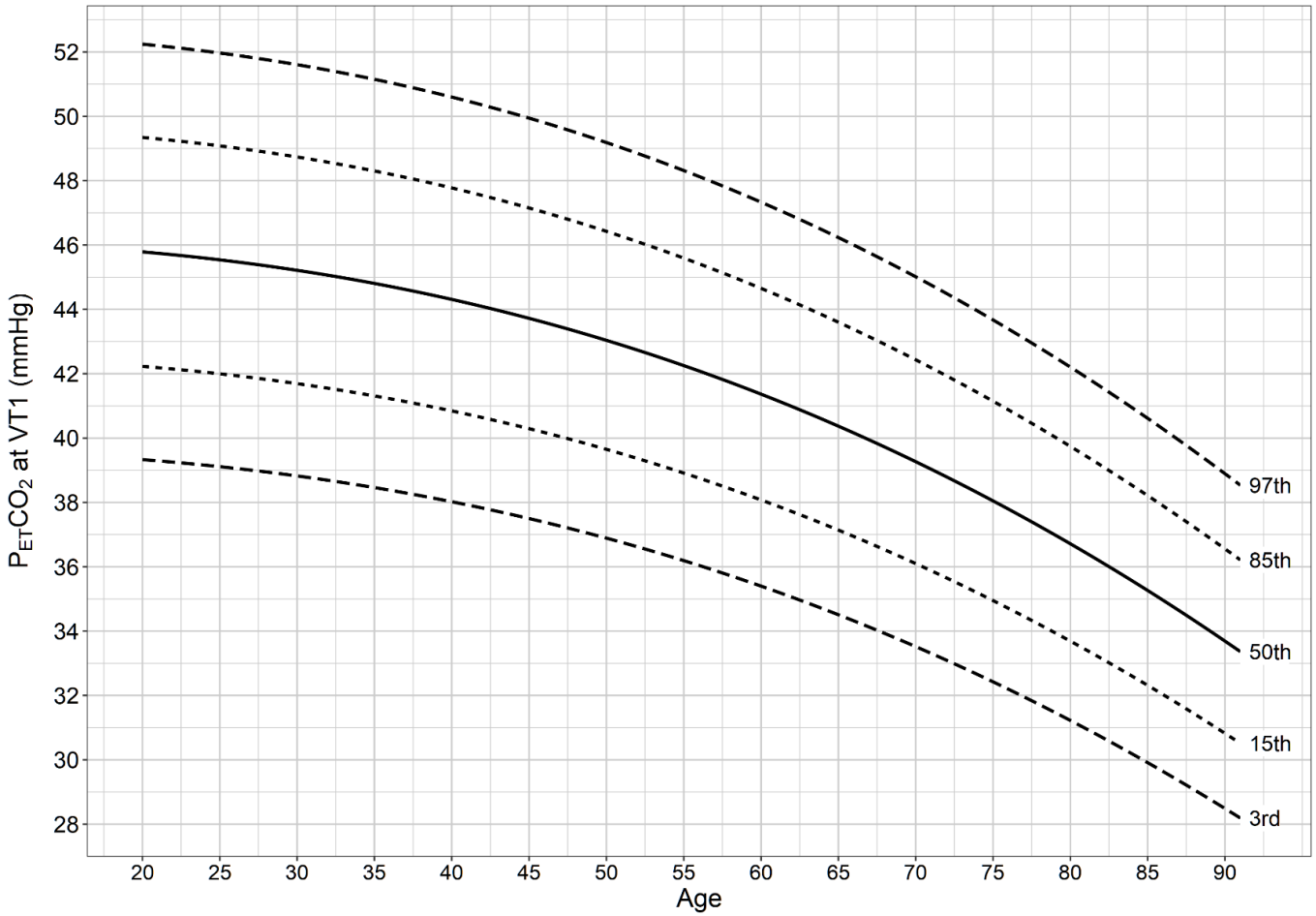
Abbreviation: $P_{ET}CO_2$, partial pressure of end-tidal CO_2

Figure SDC 1.10: Quantile curves for women for $P_{ET}CO_2$ at rest (mmHg)



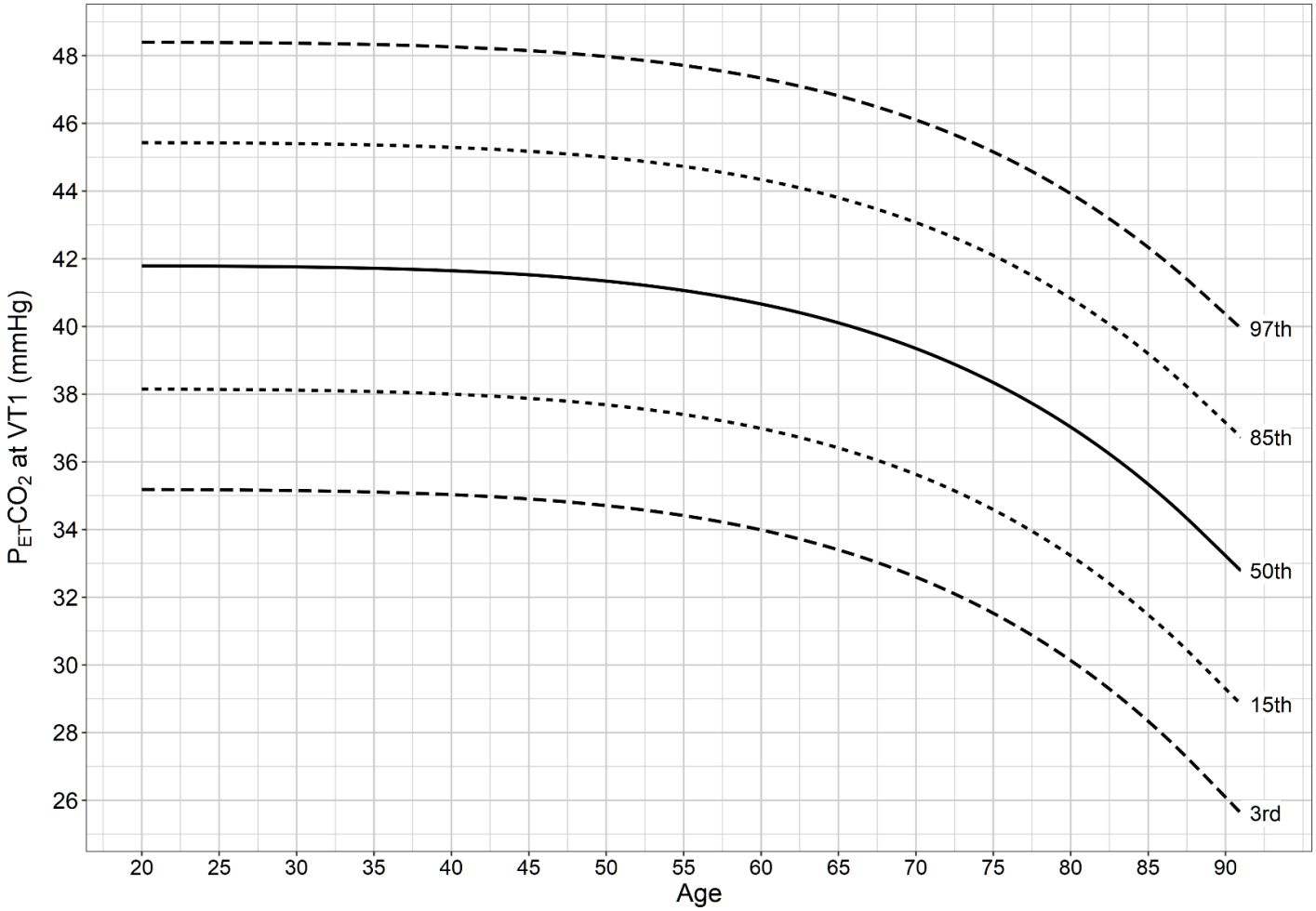
Abbreviation: $P_{ET}CO_2$, partial pressure of end-tidal CO_2

Figure SDC 1.11: Quantile curves for men for $P_{ET}CO_2$ at VT1 (mmHg)



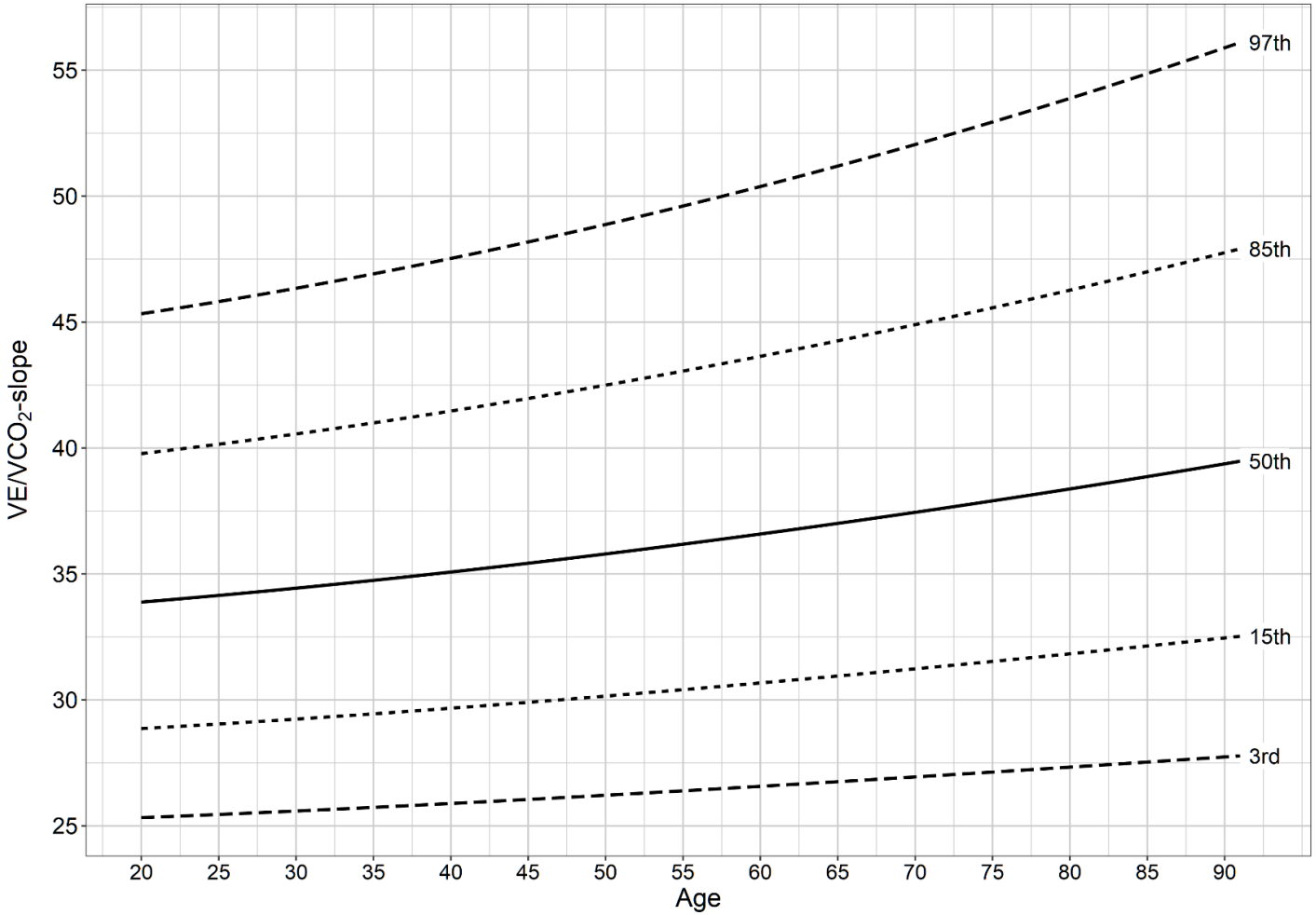
Abbreviations: $P_{ET}CO_2$, partial pressure of end-tidal CO_2; VT1, ventilatory threshold 1

Figure SDC 1.12: Quantile curves for women for $P_{ET}CO_2$ at VT1 (mmHg)



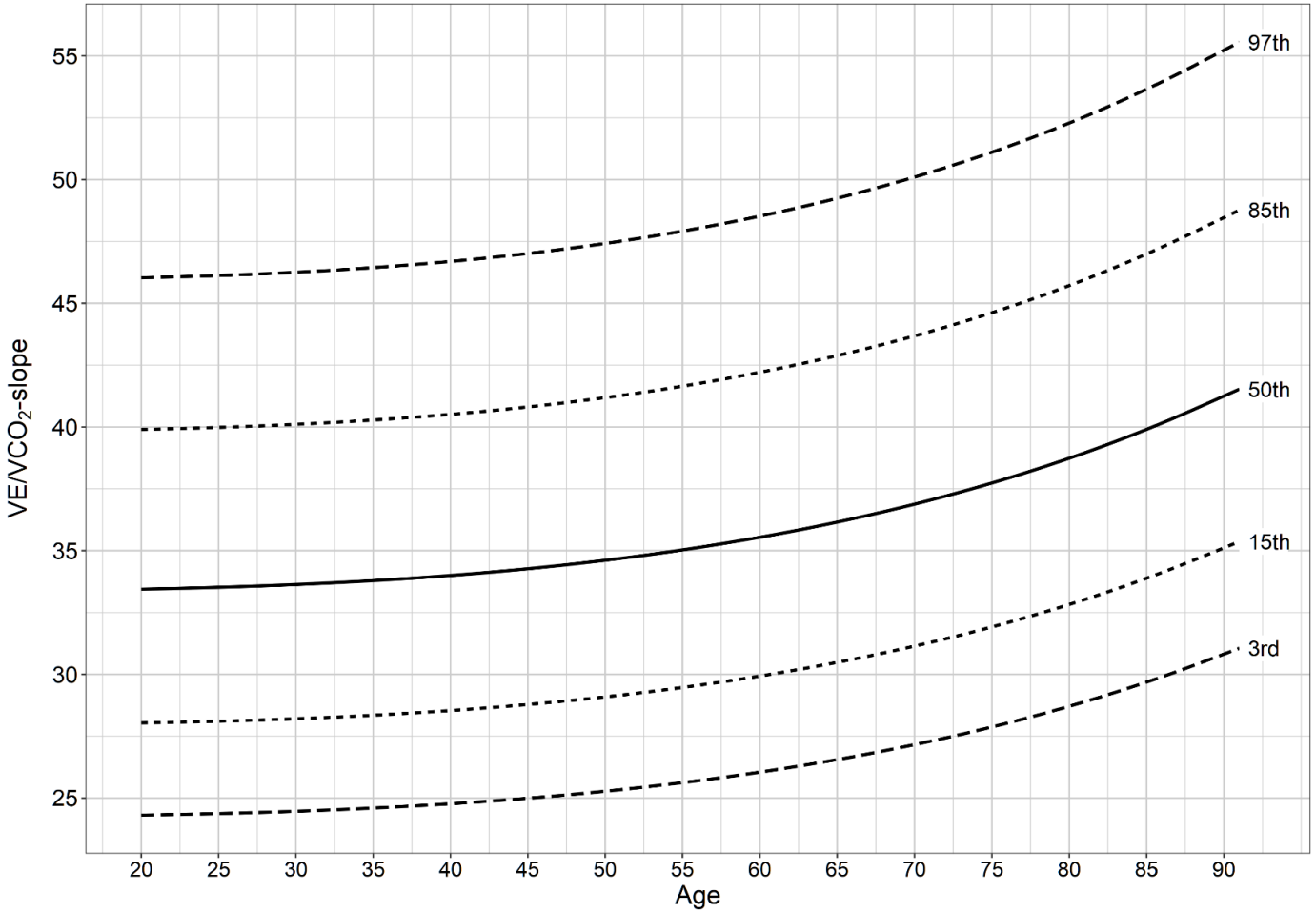
Abbreviations: $P_{ET}CO_2$, partial pressure of end-tidal CO_2; VT1, ventilatory threshold 1

Figure SDC 1.13: Quantile curves for men for $\dot{V}_E/\dot{V}CO_2$ slope



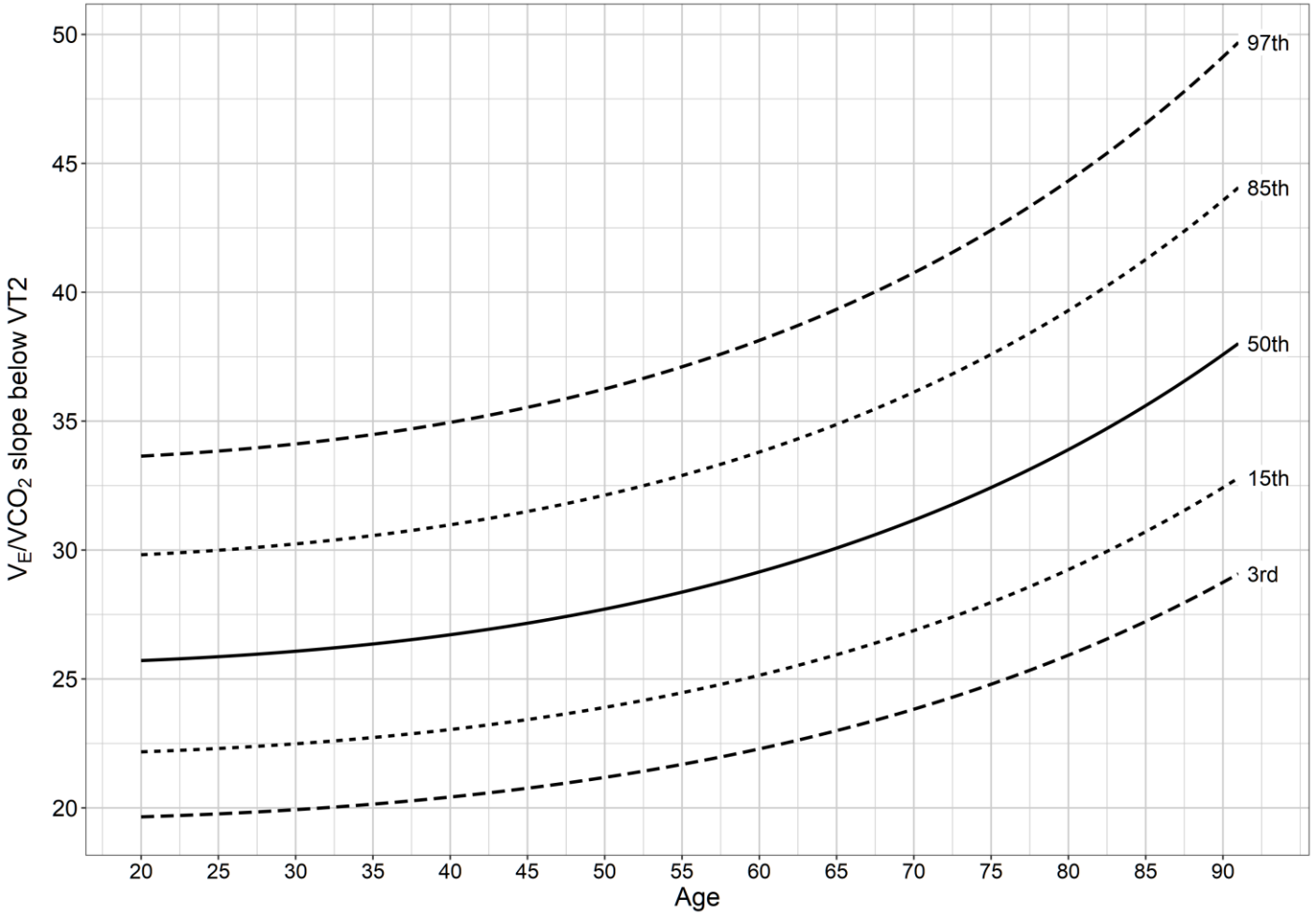
Abbreviations: $\dot{V}_E$, volume of expiration; $\dot{V}CO_2$, carbon dioxide output

Figure SDC 1.14: Quantile curves for women for $\dot{V}_E/\dot{V}CO_2$ slope



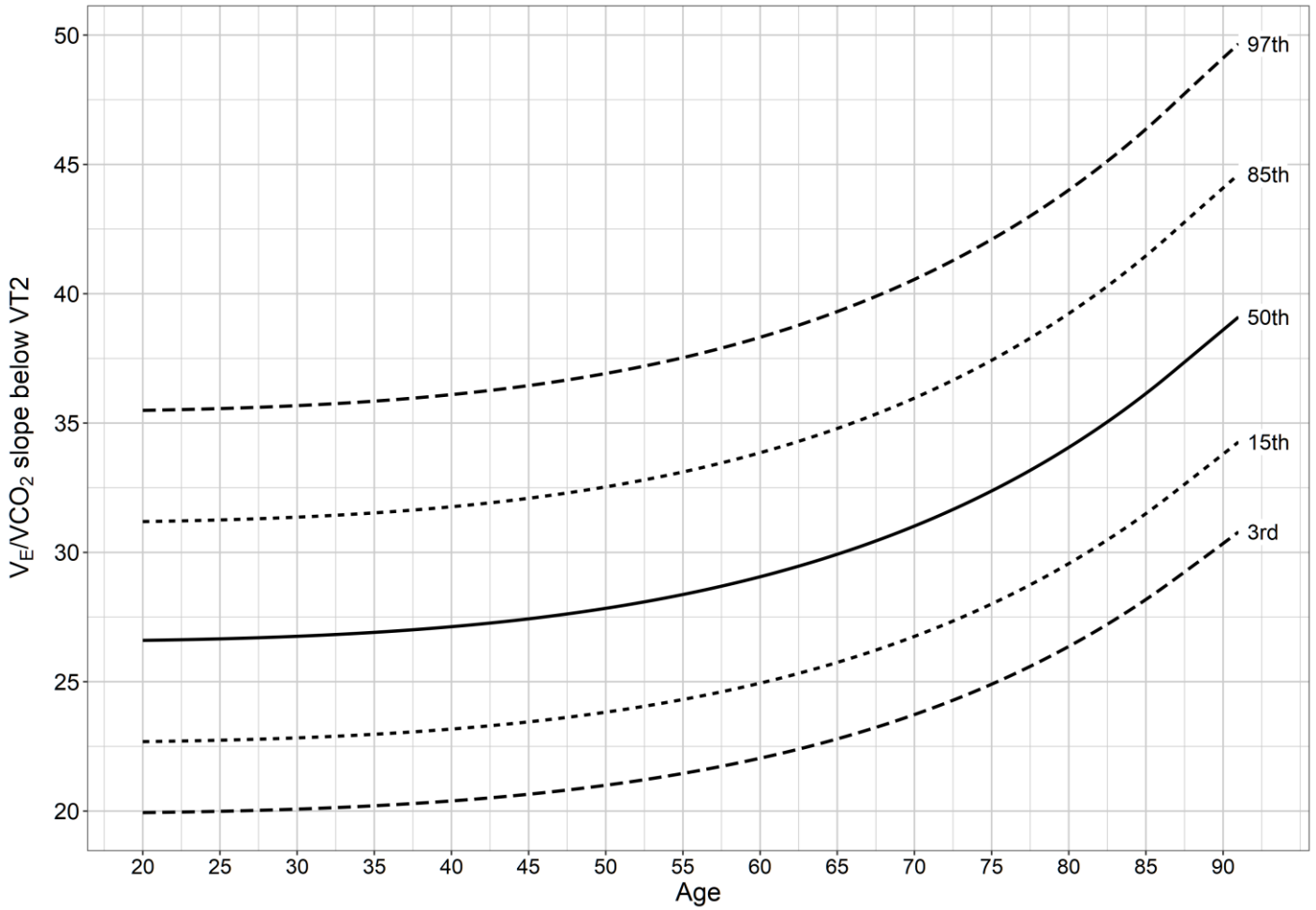
Abbreviations: $\dot{V}_E$, volume of expiration; $\dot{V}CO_2$, carbon dioxide output

Figure SDC 1.15: Quantile curves for men for $\dot{V}_E/\dot{V}CO_2$ slope below VT2



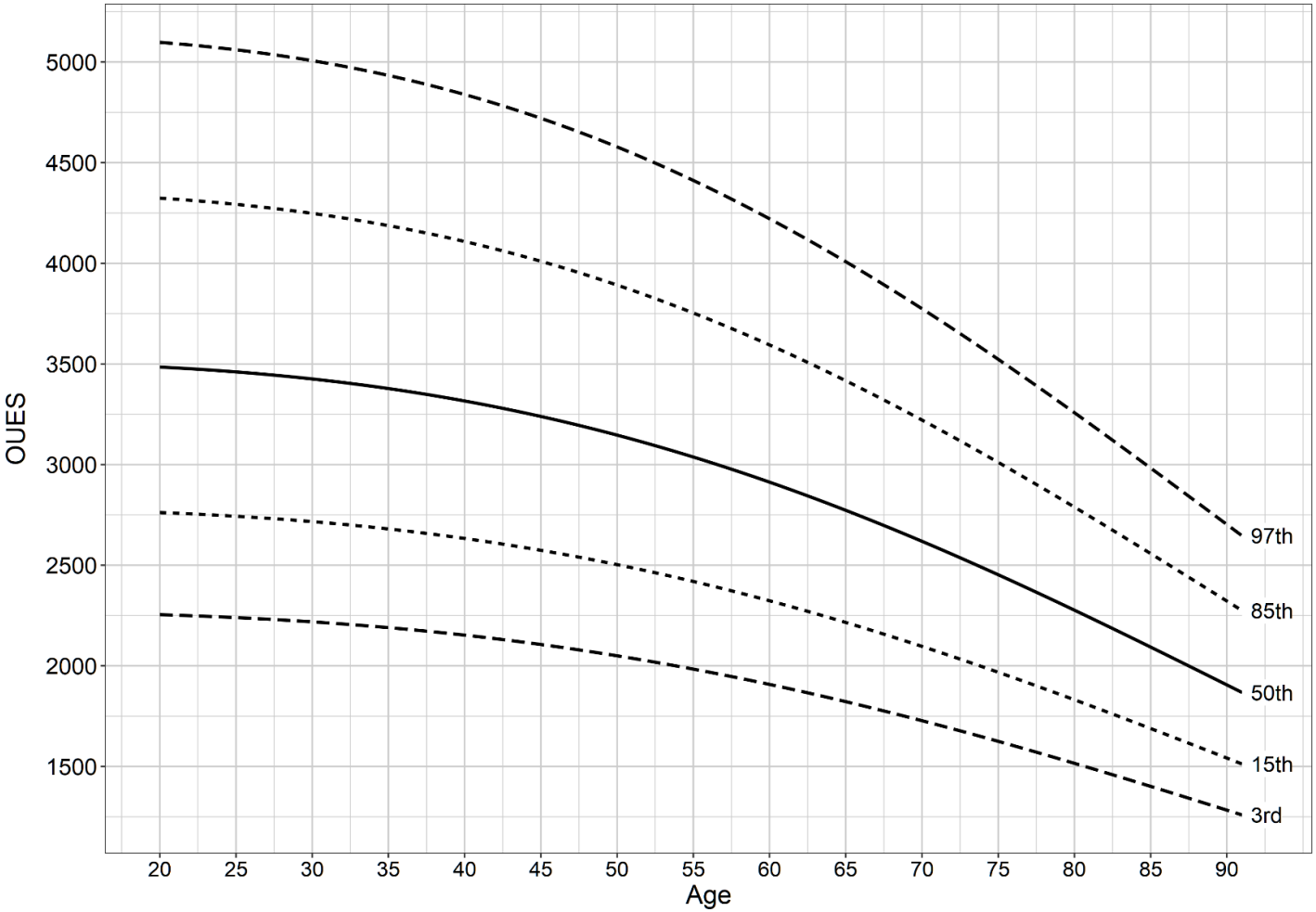
Abbreviations: $\dot{V}_E$, volume of expiration; $\dot{V}CO_2$, carbon dioxide output; VT2, ventilatory threshold 2

Figure SDC 1.16: Quantile curves for women for $\dot{V}_E/\dot{V}CO_2$ slope below VT2



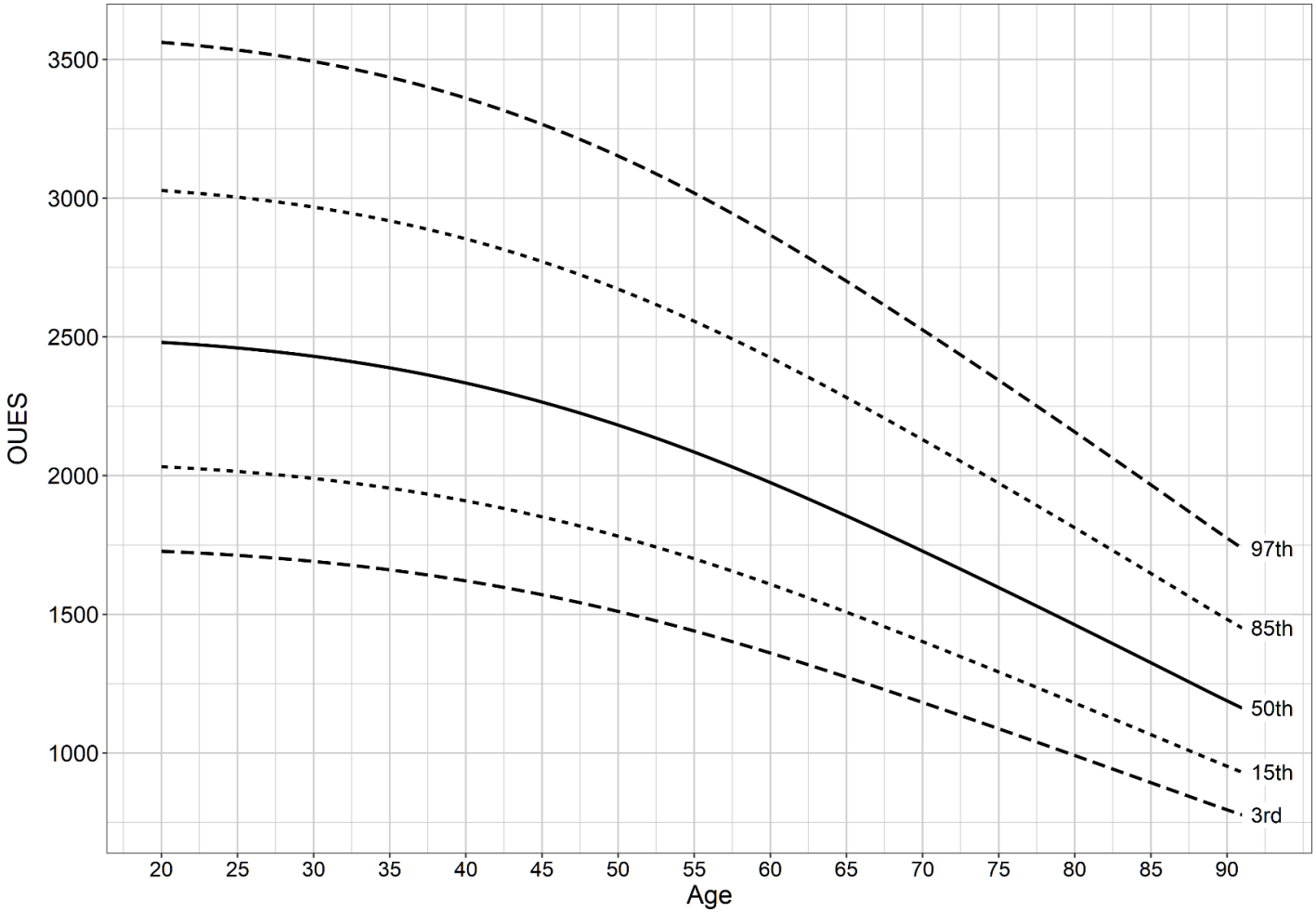
Abbreviations: $\dot{V}_E$, volume of expiration; $\dot{V}CO_2$, carbon dioxide output; VT2, ventilatory threshold 2

Figure SDC 1.17: Quantile curves for men for OUES (mL/min)



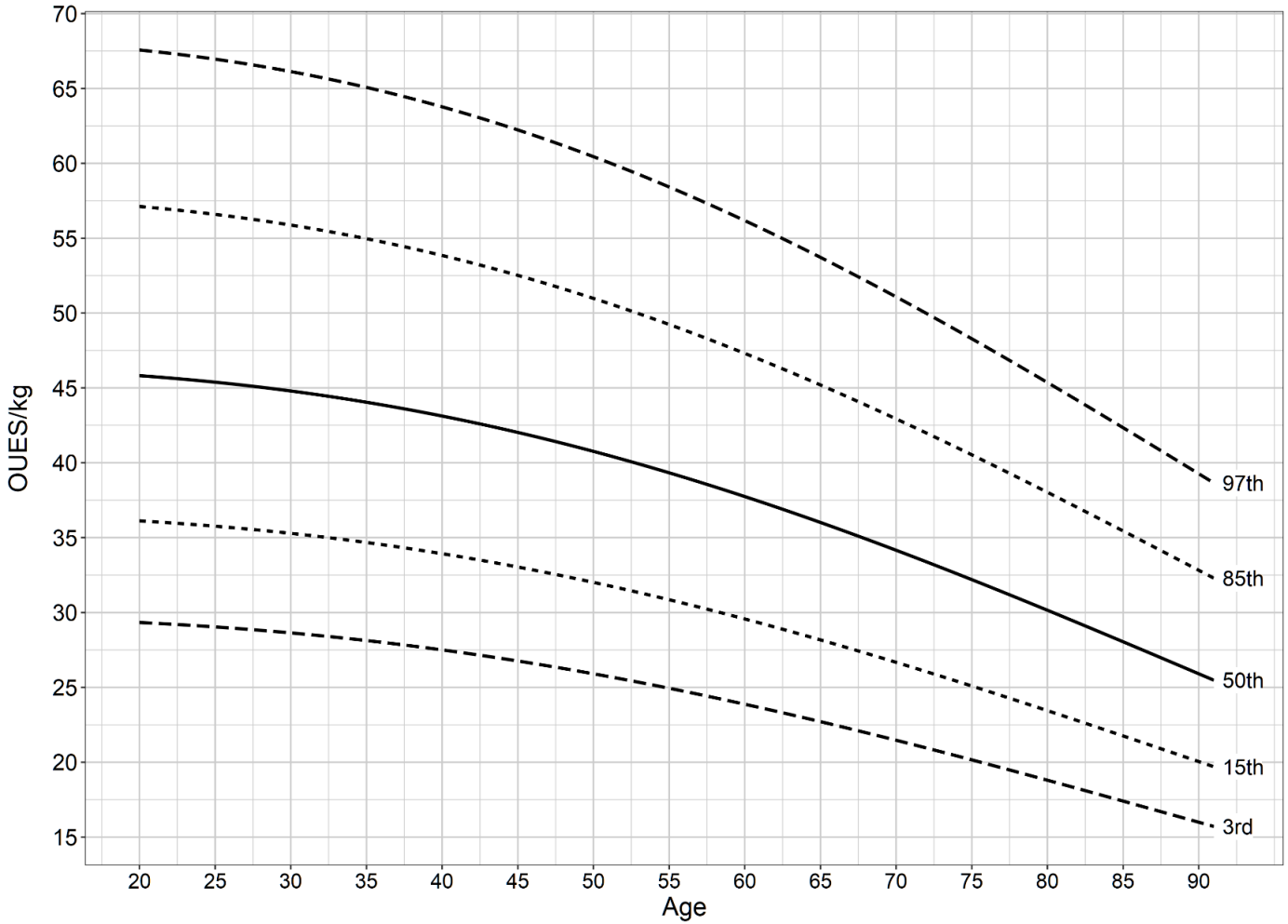
Abbreviation: OUES, oxygen uptake efficiency slope

Figure SDC 1.18: Quantile curves for women for OUES (mL/min)



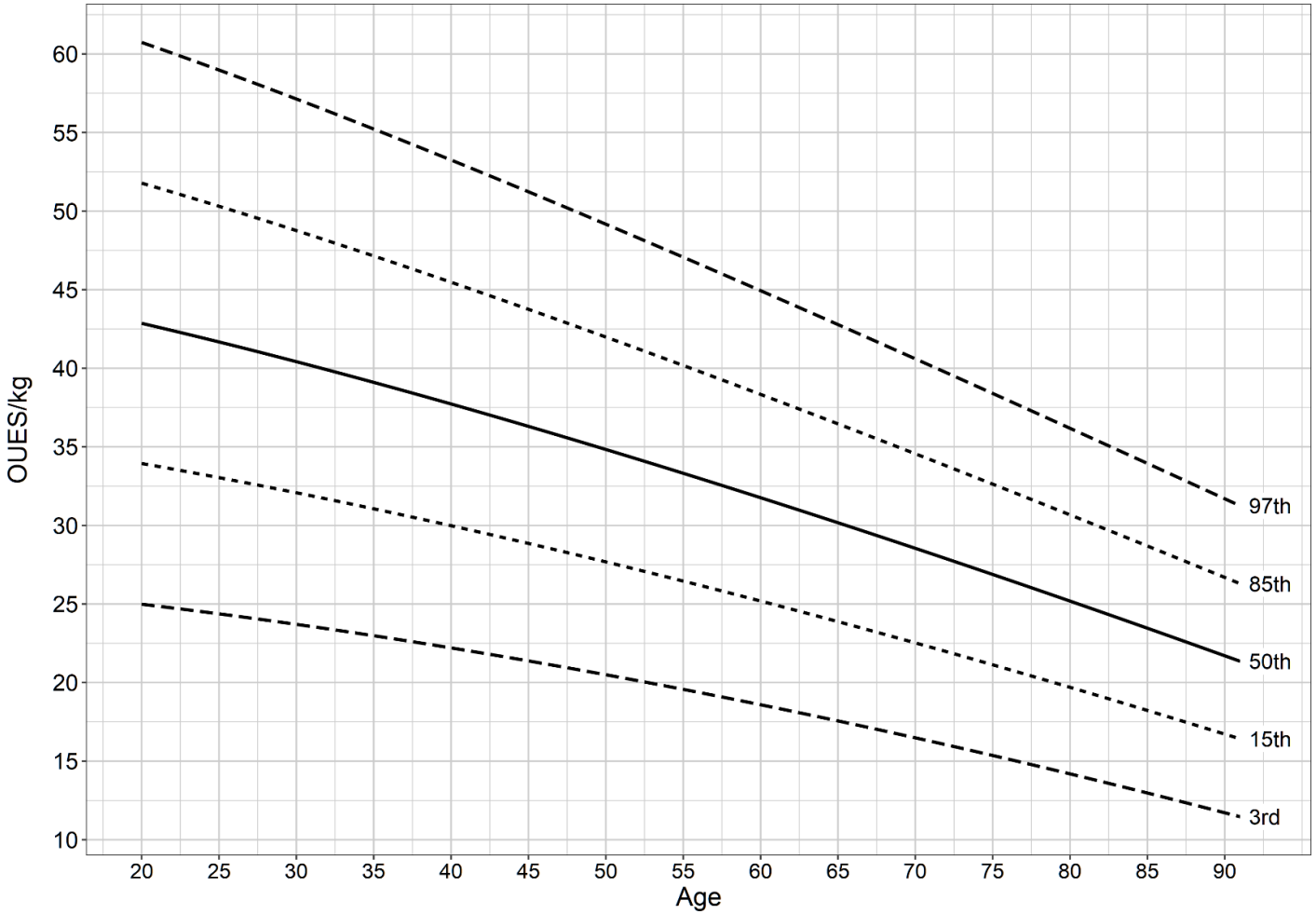
Abbreviation: OUES, oxygen uptake efficiency slope

Figure SDC 1.19: Quantile curves for men for OUES (mL/min/kg)



Abbreviation: OUES, oxygen uptake efficiency slope

Figure SDC 1.20: Quantile curves for women for OUES (mL/min/kg)



Abbreviation: OUES, oxygen uptake efficiency slope

Figure SDC 1.21: Quantile curves for men for peak heart rate (beats/min)

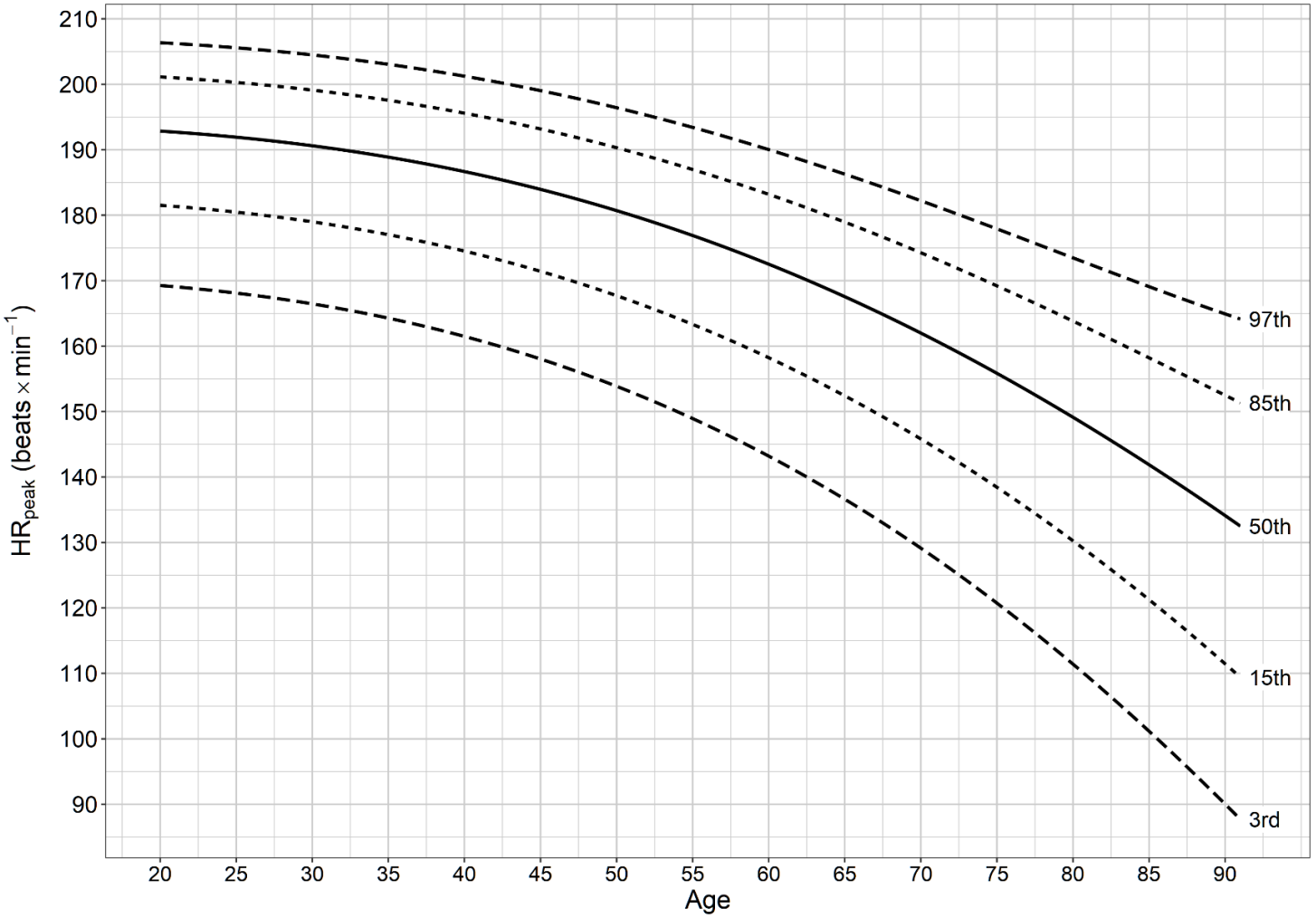


Figure SDC 1.22: Quantile curves for women for peak heart rate (beats/min)

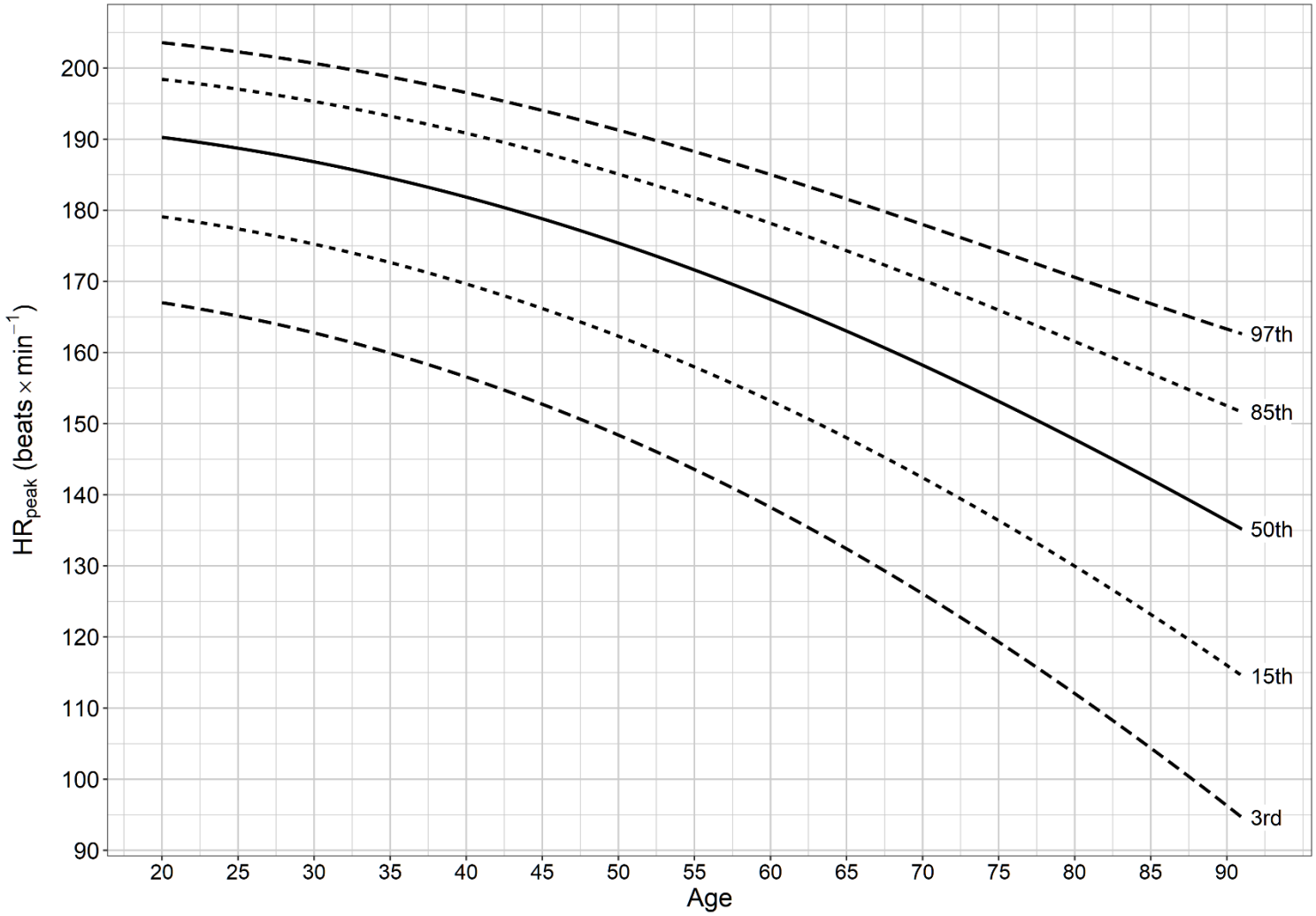
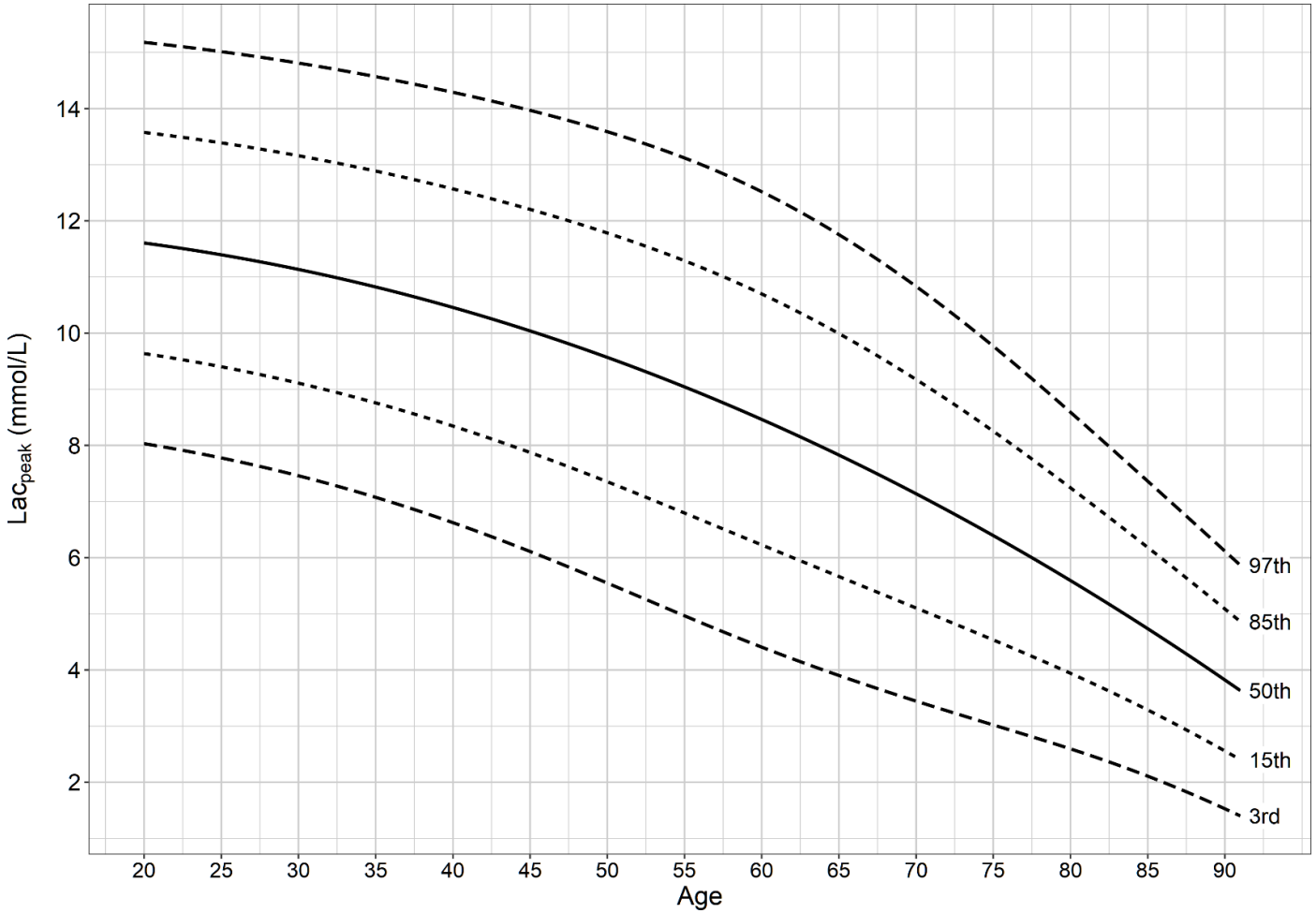
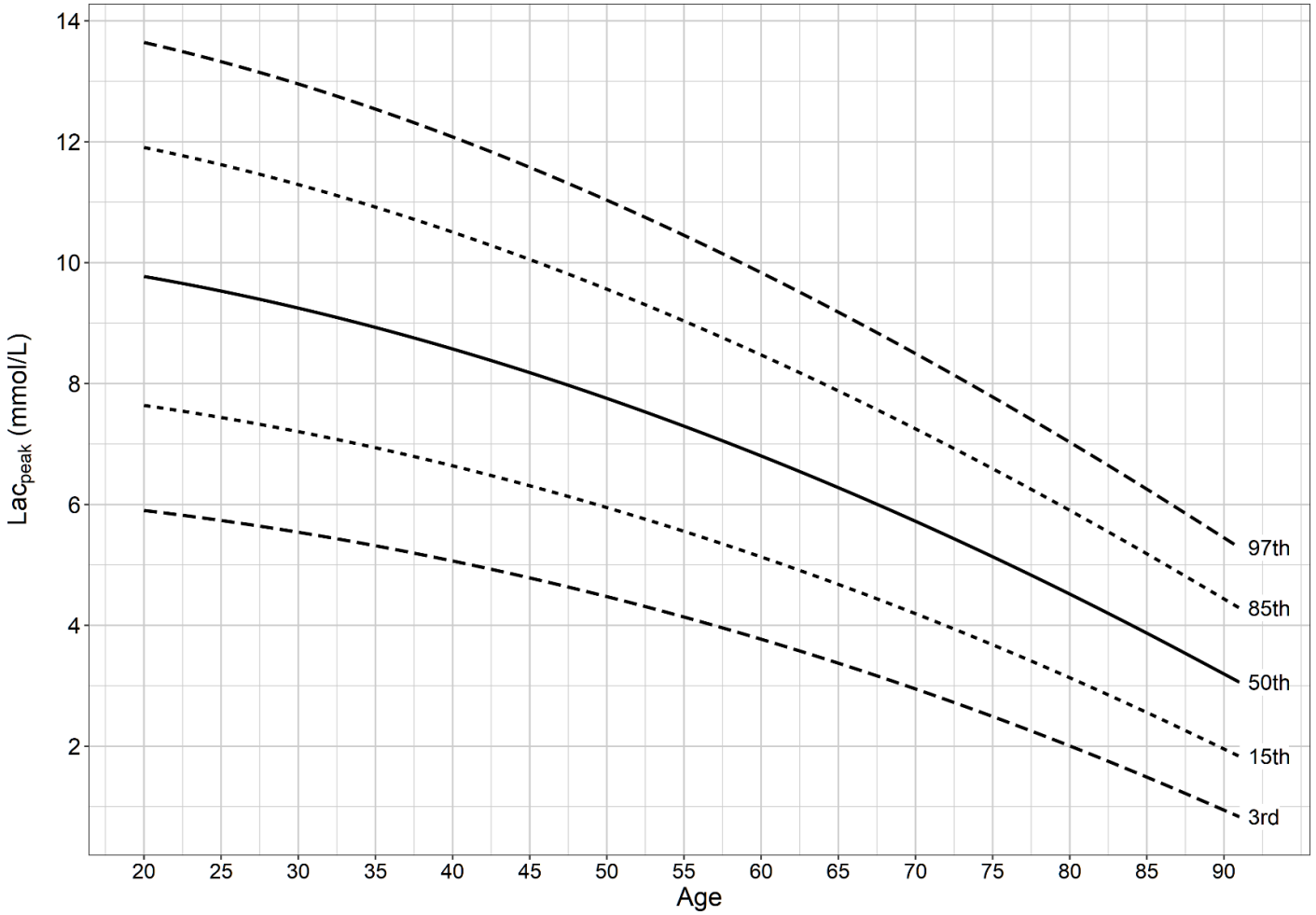


Figure SDC 1.23: Quantile curves for men for peak lactate (mmol/L)



Abbreviation: Lac, lactate.

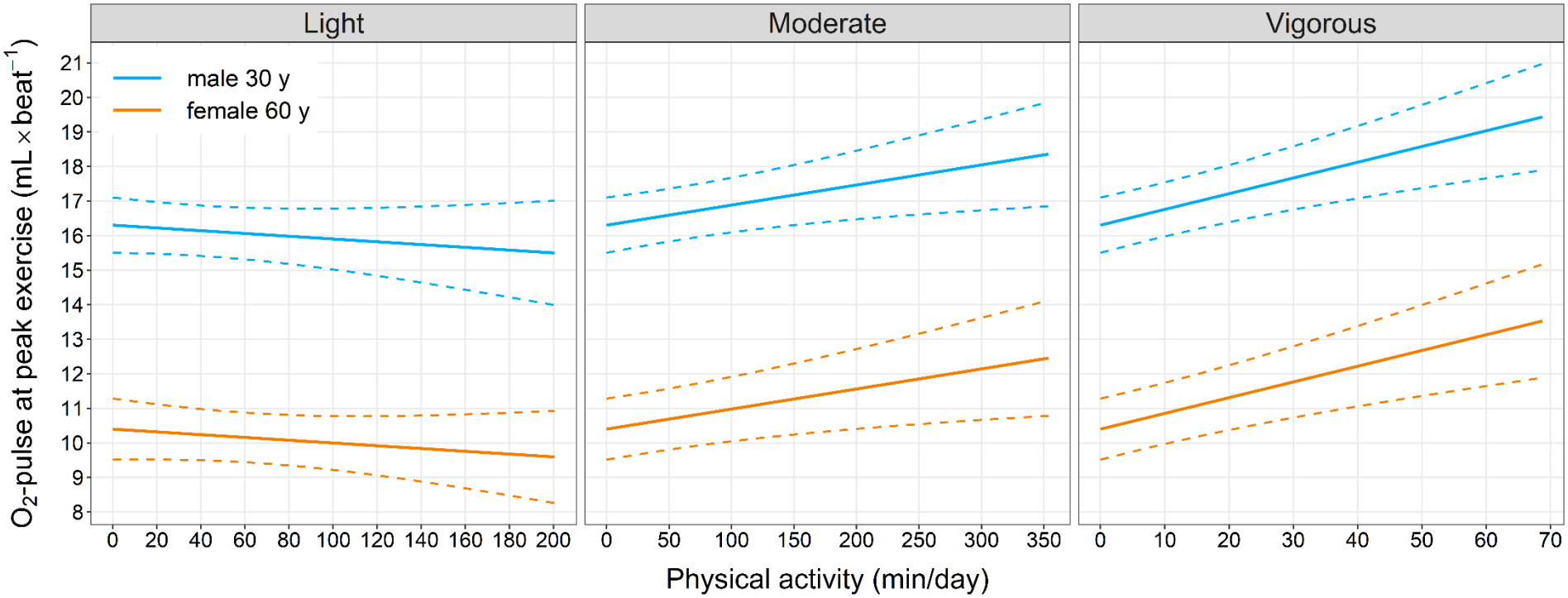
Figure SDC 1.24: Quantile curves for women for peak lactate (mmol/L)



Abbreviation: Lac, lactate.

SDC 2: Association of key CPET parameters and physical activity

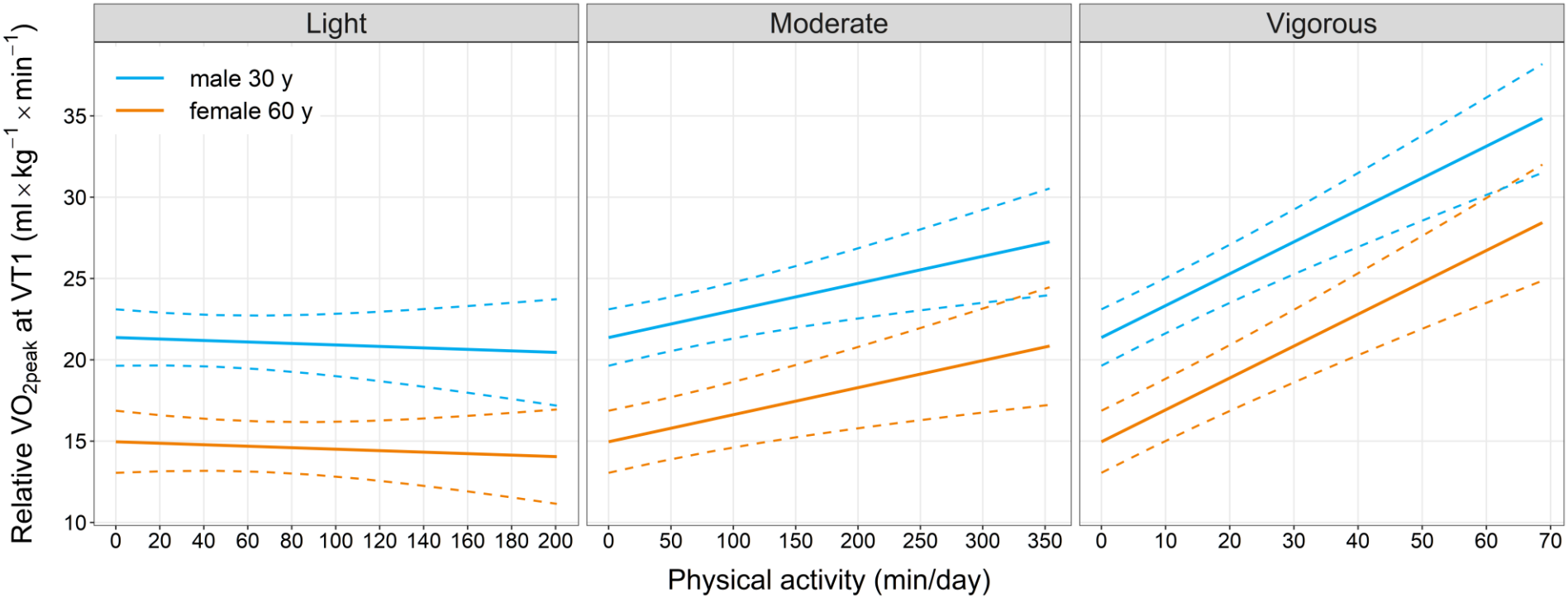
Figure SDC 1.1: Associations of LPA, MPA, and VPA and Peak O_2pulse (mL/beat)



To visualize the associations between Peak O_2pulse and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Abbreviations: LPA, light physical activity, MPA, moderate physical activity, VPA, vigorous physical activity.

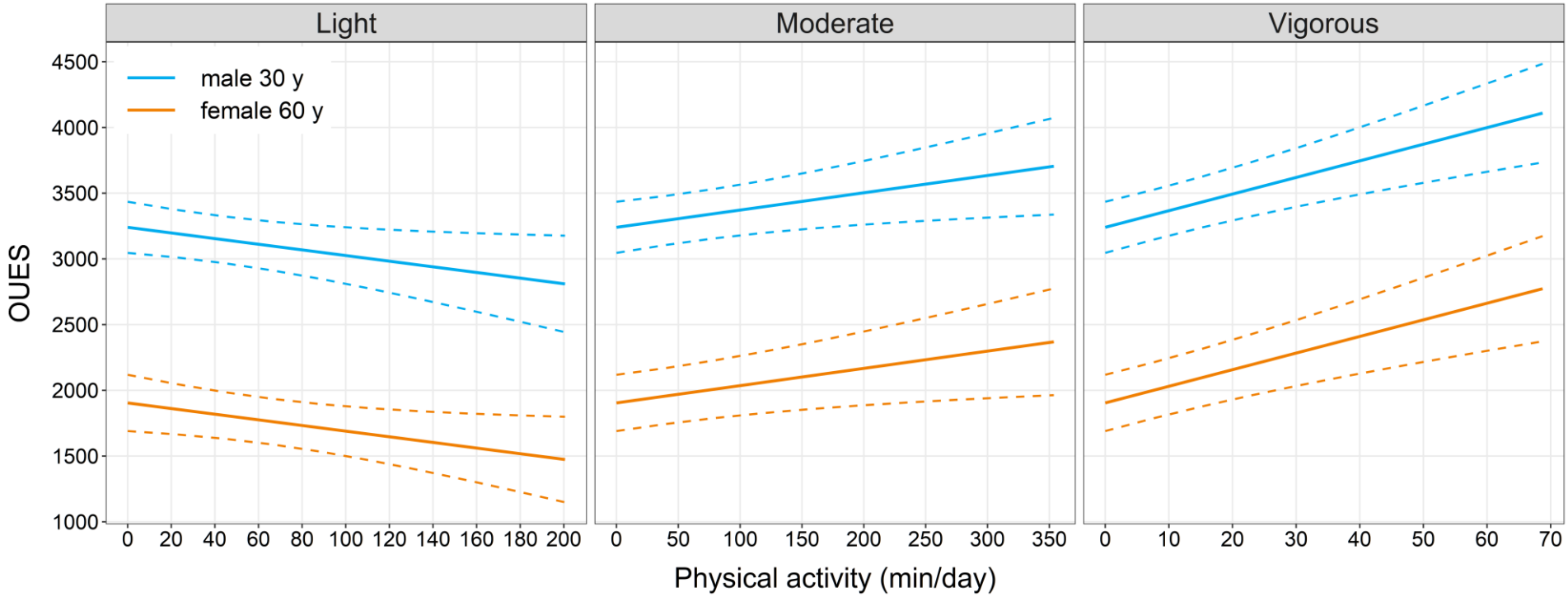
Figure SDC 1.2: Associations of LPA, MPA, and VPA and relative $\dot{V}O_{2peak}$ at VT1



To visualize the associations between relative $\dot{V}O_{2peak}$ at VT1 and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Abbreviations: LPA, light physical activity, MPA, moderate physical activity, VPA, vigorous physical; $\dot{V}O_2$, oxygen uptake; VT1, ventilatory threshold 1.

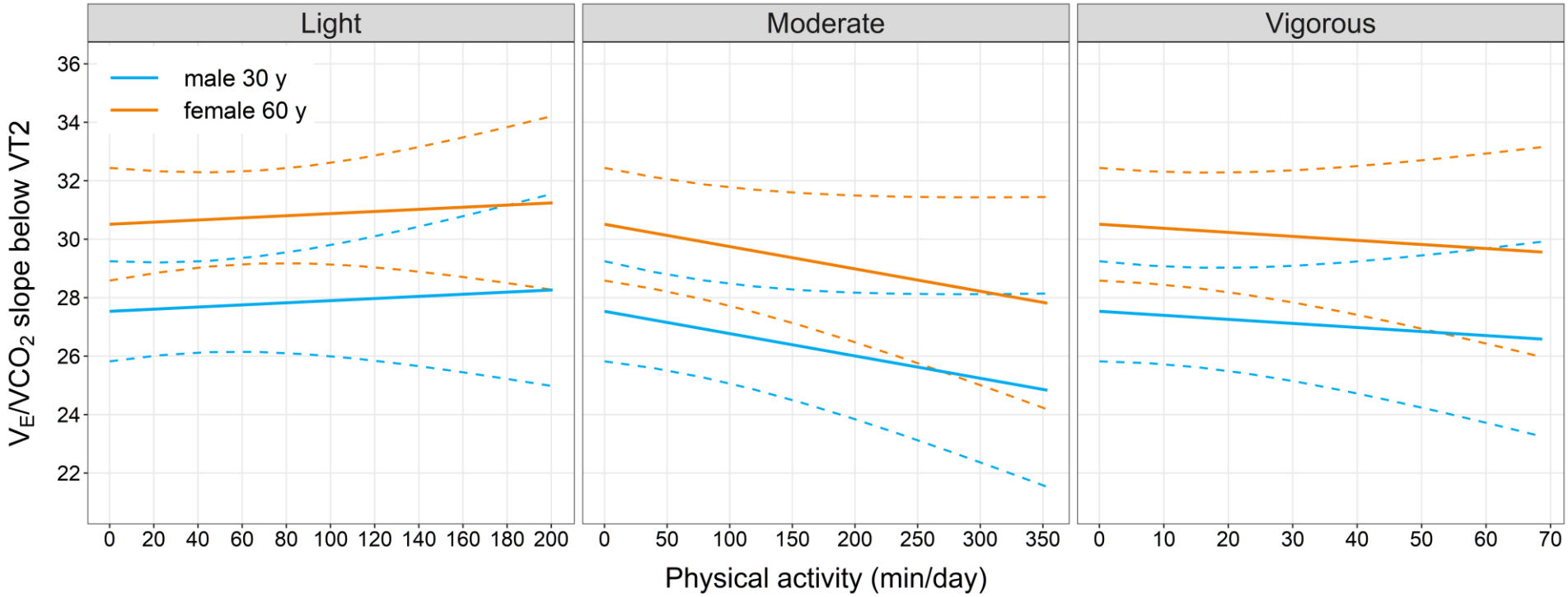
Figure SDC 1.3: Associations of LPA, MPA, and VPA and OUES (mL/min)



Legend Figure 27: To visualize the associations between OUES and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Abbreviations: LPA, light physical activity, MPA, moderate physical activity, VPA, vigorous physical; OUES, oxygen uptake efficiency slope**.**

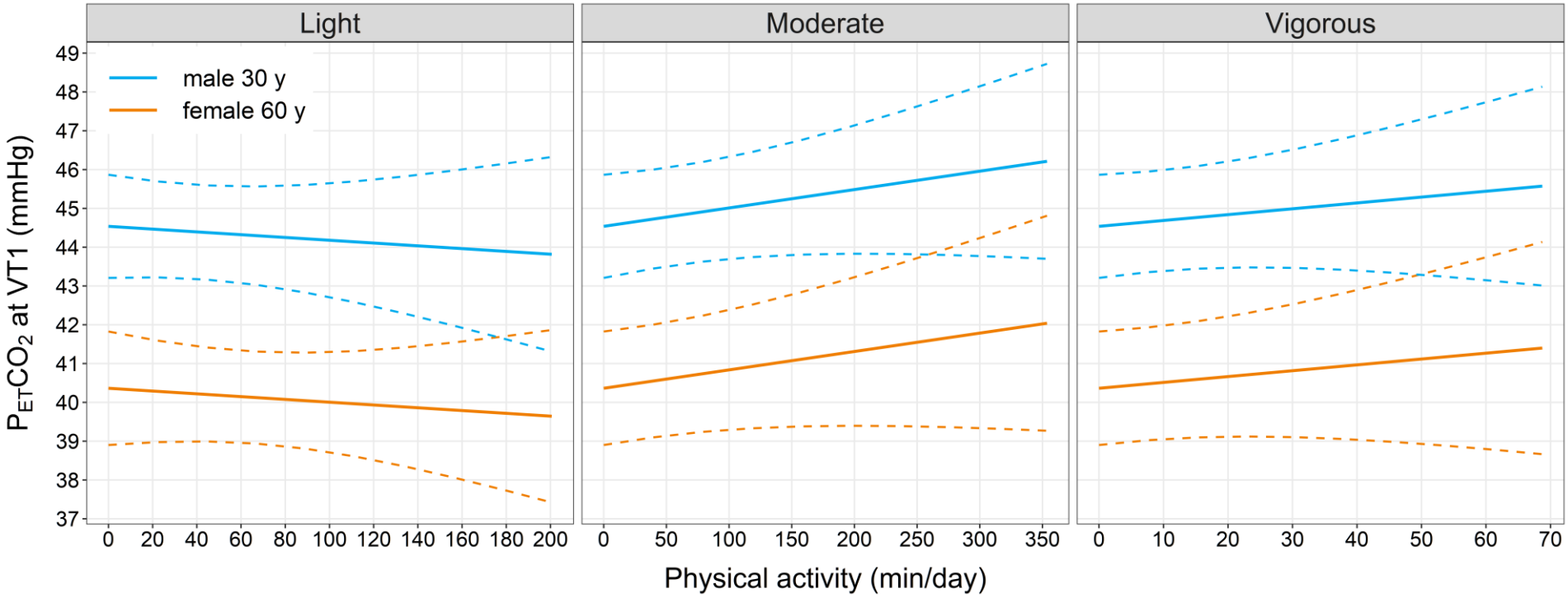
Figure SDC 1.4: Associations of LPA, MPA, and VPA and $\dot{V}_E/\dot{V}CO_2$ slope below VT2



Legend Figure 28: To visualize the associations between $\dot{V}_E/\dot{V}CO_2$ slope below VT2 and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Abbreviations: LPA, light physical activity, MPA, moderate physical activity, VPA, vigorous physical; $\dot{V}_E$, volume of expiration; $\dot{V}CO_2$, carbon dioxide output; VT2, ventilatory threshold 2.

Figure SDC 1.5: Associations of LPA, MPA, and VPA and $P_{ET}CO_2$ at VT1.



To visualize the associations between $P_{ET}CO_2$ at VT1 and levels of LPA, MPA, and VPA, exemplary values for the independent variables age and sex were inserted into the linear regression.

Abbreviations: LPA, light physical activity, MPA, moderate physical activity, VPA, vigorous physical; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; VT1, ventilatory threshold 1.

SDC 3: Methodological quality list according to Takken et al. 2019 and the ATS/ACCP guidelines for CPET reference values

		Criteria	Fulfillment of the criteria by COmPLETE-Health (0 = no, 1 = yes)
1	Population characteristics	Subjects are community based.(The subjects studied preferably be community bases rather than hospital based).	1
2		Level of physical activity is reported.	1
3		Exclusion of different racial groups	1
4		Exclusion of smokers in the sample studied	1
5		No lack of definition of de confidence limits for individual or specified characteristics.(Include age, sex, and anthropomorphic considerations).	1
6	Sample size	The number of subjects tested is sufficiently equal or larger than the appropriately powered sample size, with a uniform distribution of subjects for sex and groups.(Specific attention is given to include women and older individuals, given the changing demographics and paucity of reliable population-based CPET data for these groups).	1
7	Randomization	Randomization was applied.(The study design includes a randomization process to avoid the potential bias seen when more physically active subjects volunteer for the study).	1/0 Randomization was performed. Due to the relatively low response rate to the recruitment letters, a potential bias could still be prevalent.
8	Design	A prospective study design	1
9	Quality assurance of equipment and methodologies	Quality control was applied.(Quality was achieved using recommendations contained in the ATS/ACCP guidelines and the CPET protocols in accordance with recommendations specified in the ATS/ACCP guidelines).	1
10		Exercise testing protocol and procedures are described.	1
11		Results are obtained by either breath-by-breath analysis or mixing chamber treated in accordance with recommendation contained in the ATS/ACCP guidelines.	1
12	Treatment of data	CPET result in interval averaged, preferably every 30–60 s (to avoid the noise of shorter interval), and the peak value reported represents the mean of the last-completed stage or of all the data collected during the final stage, but preferably for no less than 30 s.	1
13	Validation	Reference equations are validated in population other than those used to generate the existing data	0
14	Statistical treatment of data	The function that most accurately describes the distribution of the data are used. For example, curvilinear (power) functions may more accurately describe the distribution of the data. Furthermore, the precision of the individual and population predicted values are reported.	1
Total score			**12**

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Chapter 7

Publication 5

VO_2 Kinetics: An Alternative to Peak VO_2 for Risk Stratification and Diagnosis in Heart Failure

Authors:

Jonathan Wagner[1]*, Max Niemeyer[2]*, Denis Infanger[1], Otmar Pfister[3], Jonathan Myers[4], Arno Schmidt-Trucksäss[1]*, Raphael Knaier[1]*

*shared First Authorship
*shared Senior Authorship

[1] Department of Sport, Exercise and Health, Division Sports and Exercise Medicine, University of Basel, Basel, Switzerland
[2] Department Medicine, Training and Health, Institute of Sport Science and Motology, Philipps-University Marburg, Marburg, Germany
[3] Department of Cardiology, University Hospital Basel and University of Basel, Basel, Switzerland
[4] Cardiology Division, VA Palo Alto Health Care System and Stanford University, Palo Alto, CA, USA

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Abstract

Background

To analyse whether $\dot{V}O_2$-kinetics during cardiopulmonary exercise testing (CPET) is a useful marker for the diagnosis of heart failure and to determine which $\dot{V}O_2$-kinetic parameter distinguishes healthy participants and patients with heart failure (HF).

Methods

526 healthy participants and 79 patients with HF between 20 and 90 years of age performed a CPET. The CPET was preceded by a 3-minute low-intensity warm-up and followed by a 3-minute recovery bout. $\dot{V}O_2$-kinetics was calculated from the rest to exercise transition of the warm-up bout (on-kinetics), from the exercise to recovery transition following ramp test termination (off-kinetics) and from the initial delay of $\dot{V}O_2$ during the warm-up to ramp test transition (ramp-kinetics).

Results

$\dot{V}O_2$ off-kinetics showed the highest z-score differences between healthy participants and patients with HF. Furthermore, off-kinetics were strongly associated with $\dot{V}O_{2peak}$. In contrast, ramp-kinetics and on-kinetics showed only minimal z-score differences between healthy participants and patients with HF. The best on- and off-kinetic parameters significantly improved a model to predict disease severity. However, there was no relevant additional value of $\dot{V}O_2$-kinetics when $\dot{V}O_{2peak}$ was part of the model.

Conclusions

$\dot{V}O_2$ off-kinetics appears to be superior for distinguishing patients with HF and healthy participants compared to $\dot{V}O_2$ on-kinetics and ramp-kinetics. If $\dot{V}O_{2peak}$ can not be determined, $\dot{V}O_2$ off-kinetics provides an acceptable substitute. However, additional value beyond that of $\dot{V}O_{2peak}$ can not be provided by $\dot{V}O_2$-kinetics.

Introduction

The incidence and prevalence of heart failure (HF) are high and continue to increase in the developed world with ageing of the population. Concomitant deaths and health care costs related to this syndrome are increasing (1). Accurate diagnostic and risk assessment methods for HF are essential to guide clinical decisions for therapeutic strategies with the ultimate goal of decreasing risk and improving health outcomes. Gas exchange variables obtained through cardiopulmonary exercise testing (CPET) are an established method for accurately stratifying risk in HF patients; many CPET responses now have a substantial evidence base (2). However, maximal CPET parameters can be difficult to interpret as they are highly dependent on subject effort (3). As many HF patients are not familiar with severe exercise intensities, pushing these patients to their physiological limit remains a challenge. Therefore, there have been many efforts to investigate submaximal markers such as oxygen uptake kinetics ($\dot{V}O_2$-kinetics) in patients with HF.

$\dot{V}O_2$-kinetics represent the rate at which aerobic ATP generation adjusts to changes in exercise intensity (4). This parameter is dependent on the cardiovascular system's ability to rapidly increase or decrease the oxygen supply to the working muscles (5, 6) as well as by the ability of the muscles to rapidly utilise oxygen (7). Therefore, $\dot{V}O_2$-kinetics can provide critical information regarding the regulating capacity of the cardiovascular system and the skeletal muscles to utilize oxygen (5), and thus exercise intolerance and functional mobility (8-10).

Studies investigating whether $\dot{V}O_2$-kinetics is a useful marker for risk stratification in HF have reported conflicting findings (9-15). While some have reported that the prognostic value of $\dot{V}O_2$-kinetics is even superior to $\dot{V}O_{2peak}$ (12, 15) others have reported only moderate or minimal additional value beyond $\dot{V}O_{2peak}$ (9-11). These conflicting results are likely caused by the fact that varying features of $\dot{V}O_2$-kinetics were analysed (i.e. on-kinetics or i.e. off-kinetics) and different calculation approaches were used.

$\dot{V}O_2$-kinetics is traditionally measured by performing a constant load test. CPET using a ramp protocol is however, the preferred method to perform an exercise test in the clinical setting (16). This manuscript therefore focused only on the utility of $\dot{V}O_2$-kinetics during a ramp protocol.

The aims of the study were: 1) to analyse whether $\dot{V}O_2$-kinetics parameters obtained from a CPET can distinguish between healthy participants and cardiac patients with HF and between NYHA functional classes; 2) to determine which $\dot{V}O_2$-kinetic parameter and which calculation is most valid; and 3) whether the most promising $\dot{V}O_2$ on- and $\dot{V}O_2$ off-kinetic parameter can add additional value to $\dot{V}O_{2peak}$.

Methods

Study design

Population and recruitment

The COmPLETE-Study is a cross-sectional single-centre study and consists of two parts, COmPLETE-Health and COmPLETE-Heart. COmPLETE-Health included healthy men and women without any known exercise limiting diseases between 20 and 90 years of age equally distributed across age decades and sex. COmPLETE-Heart included cardiac patients with stable HF with NYHA functional class I-III, with symptoms and signs stable for at least one month. Diagnosis of HF was confirmed by clinical history, physical examination, assessment of natriuretic peptide (NT-proBNP) and echocardiographically-documented structural heart disease or diastolic dysfunction according to the European Society of Cardiology guidelines (17). Details on recruitment procedures and complete inclusion and exclusion criteria can be found in the study protocol (18).

Setting

The study was carried out at the Department of Sport, Exercise, and Health at the University of Basel, Switzerland and was funded by the Swiss National Science Foundation (grant no. 182815). The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451). Written informed consent has been obtained from all participants.

Acquisition of participant characteristics

Resting systolic and diastolic blood pressures were measured with the participant in the supine position using a noninvasive vascular screening system (VaSera VS-1500 N; Fukuda Denshi, Tokyo, Japan). Physicians assessed medical history and medications by questionnaire onsite. Based on clinical data, structured questions and self-reported exercise tolerance, each patient with HF was assigned to a NYHA functional class by a physician who was blinded to both CPET results and laboratory data. Blood samples were drawn by venipuncture by trained medical staff in fasting state (at least three hours). Samples were immediately centrifuged, the plasma aliquots were frozen at a temperature of −80 °C and all samples together analyzed after completion of the study.

Cardiopulmonary exercise testing

An exercise test to maximal voluntary exertion using an electromagnetically braked cycle ergometer (Ergoselect 200; Ergoline, Bitz, Germany) was performed according to one of the following five ramp protocols: i) a three-minute warm-up either unloaded, a load of 10 or 20 W for protocols 1 to 3, or a load of 50 W for protocols 4 and 5 followed by ii) a ramp protocol with a linear workload increases of 7, 10, 15, 20, or 30 W/min for protocols 1 to 5 respectively, followed by iii) a three-minute recovery phase at the same workload as the warm-up. The protocol was chosen to achieve a duration of approximately 10 minutes.

Gas exchange and ventilatory variables were analyzed breath-by-breath continuously using a computer-based system (MetaMax 3B; Cortex Biophysik GmbH, Leipzig, Germany). Each test was preceded by a resting period of three minutes to reach steady-state conditions. In the absence of clinical symptoms or electrocardiographic abnormalities, all tests were continued until maximal exertion (i.e., volitional exertion, dyspnea, or fatigue). Before and during the test, healthy participants and patients with HF were verbally encouraged to reach maximal exhaustion. Before each test, the equipment was calibrated in standard fashion with reference gas and known volume.

$\dot{V}O_2$-kinetic assessment

Figure 1 displays the different methods used to determine $\dot{V}O_2$-kinetics. Initially, $\dot{V}O_2$ was filtered by removing all outliers that differed more than three standard deviations from the local mean (moving average of 6 breaths). The filtered $\dot{V}O_2$ values were then linearly interpolated to provide second-by-second values, as previously recommended (19). $\dot{V}O_2$ on-kinetics was assessed from the rest to exercise transition of the 3-minute constant load warm-up period. In accordance with previous studies we calculated the time constant of $\dot{V}O_2$ on-kinetics by two different approaches:

1) *τ $\dot{V}O_2$ on-kinetics.* A mono-exponential function was fit (see supplemental material for the exact equation) from the beginning to the end of the warm-up period using non-linear least-squares method regression analyses (10) (see Eq. 1 in the supplement).
2) *τ $\dot{V}O_2$ on-kinetics by $\dot{V}O_2$-deficit.* This was determined by the oxygen deficit and the steady-state increase of $\dot{V}O_2$ above the resting value (8, 15) (see Eq. 2 in the supplement).

$\dot{V}O_2$ off-kinetics were assessed from the active recovery period that directly followed the incremental phase of the CPET. This was done using three different approaches:

1) *τ $\dot{V}O_2$ off-kinetics.* Determined by the time constant of a mono-exponential function (see equation 3) that was fitted from the beginning to the end of the recovery period using non-linear least-squares method regression analyses (9-11) (see Eq. 3 in the supplement).
2) *slope linear $\dot{V}O_2$ off-kinetics*. Determined by the slope of a linear function that was fitted into the $\dot{V}O_2$-time relationship of the first minute of recovery using linear least-squares method regression analyses (14) (see Eq. 4 in the supplement).
3) *% rel $\dot{V}O_2$ reduction 60sec and 120sec post test.* Determined by the decrease in $\dot{V}O_2$ from the end of the incremental phase up to the first (% rel $\dot{V}O_2$ reduction 60sec post test) and second minute (% rel $\dot{V}O_2$ reduction 120sec post test) expressed as percentages of $\dot{V}O_{2peak}$ (12).

Ramp test kinetics were assessed from the initial delay of $\dot{V}O_2$ at the beginning of the incremental exercise phase (mean response time; MRT), as previously described (13, 20). For this purpose, the intersection between a horizontal line crossing the $\dot{V}O_2$ of the final 30 s of the warm-up phase ($\dot{V}O_2$ warm-up) and a straight line which was fitted into the linear $\dot{V}O_2$-work rate response of the incremental phase was calculated (see Eq. 5 in the supplement).

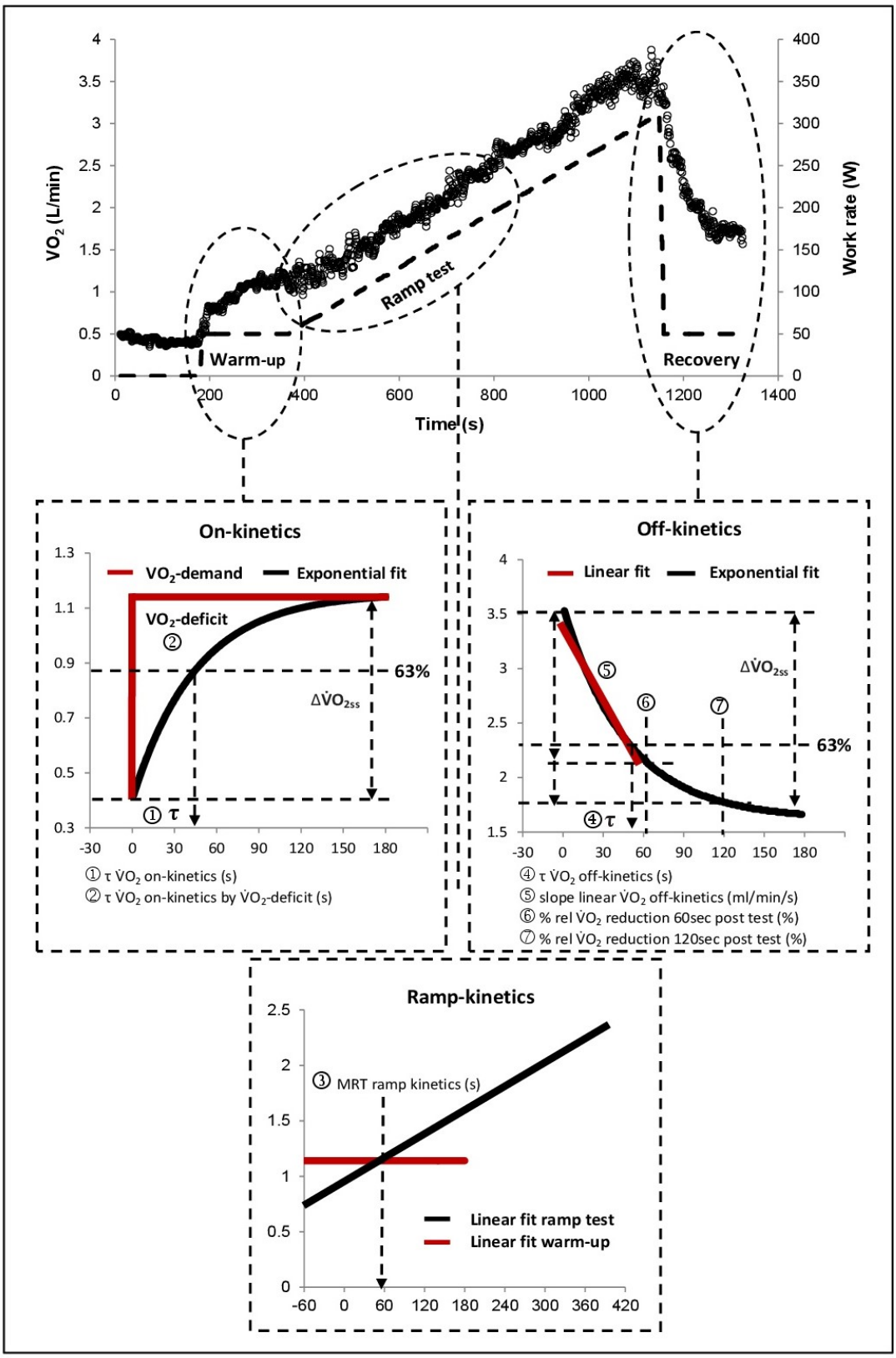

Figure 1: *Graphical illustration of all analyzed kinetic parameters*.

Statistical analysis

We investigated potential differences in $\dot{V}O_2$-kinetics variables between healthy participants and patients with heart failure using linear regression models which were adjusted for age and sex. In detail, residual diagnostics were used to see whether the model assumptions were satisfied and some kinetic parameters were subsequently log-transformed.

To investigate the associations between $\dot{V}O_2$-kinetic parameters and $\dot{V}O_{2peak}$, linear regression analyses with $\dot{V}O_{2peak}$ as the dependent variable and age, sex, and the kinetic variables as independent variables were calculated. Separate models for each kinetic parameter were built. Therefore, we modeled age using restricted cubic splines (natural splines) with four knots included along with an interaction by sex to control for potential nonlinear age progression (21). For some models, the residuals exhibited heteroscedasticity and we present robust *p*-values and confidence intervals for those models (HC3) (22).

Descriptive statistics were used to compare the $\dot{V}O_2$-kinetic variables between NYHA classes I, II, and III, and the healthy participants. To achieve comparability, we first created a matched dataset where we matched two healthy participants to every patient with HF according to age and sex (2:1 matching). We used the R package "MatchIt" for these calculations (version 3.0.2) (23).

Age- and sex-specific quantile curves were calculated using healthy participants only and applying generalized additive models for location, scale, and shape (GAMLSS, R package version 5.1-6) (24). The age-trajectories were modeled using penalized B-splines (P-splines). We adopted the Bayesian information criterion to select the conditional distribution that offered the best compromise between model complexity and goodness-of-fit. The models were inspected using diagnostic residual plots such as worm plots (25) and Q–Q plots. The *z*-scores of the patients with HF were calculated based on the established reference curves using the healthy participants.

Proportional odds ordinal logistic regressions were used to analyze whether the kinetic parameters with the largest mean difference in the *z*-scores added additional predictive information for disease severity (NYHA class). We used the unitless adequacy index to quantify the predictive information contained in $\dot{V}O_{2peak}$, age and sex compared to the full set of predictors including the kinetic parameters (21). An adequacy index near 1 indicates that $\dot{V}O_{2peak}$, age and sex contain nearly all predictive information already and that the kinetic parameters add little predictive information. We used likelihood ratio tests to assess whether the kinetic parameters improved the model fit. R version 3.6.1 or later (R Foundation for Statistical Computing, Vienna,

Austria) was used for all analyses and *p*-values ≤ 0.05 were considered statistically significant. All tests were two-sided.

Results

Participant characteristics

A total of 526 healthy participants and 79 patients with HF (NYHA functional classes I – III) were included in the study. All HF patients were in stable condition; their aetiology was cardiomyopathy (n=8), coronary artery disease (n=60), pulmonary hypertension (n=1), valvular regurgitation (n=8), and valvular stenosis (n=2). Participant characteristics are presented in Table 1.

$\dot{V}O_2$-kinetics in health and heart failure

Group differences between healthy participants and patients with HF irrespective of their NYHA class are reported in Table 2. Six out of eight $\dot{V}O_2$-kinetic parameters showed evidence for a difference between the groups ($p \leq 0.007$). The number of participants involved in the analysis of the respective kinetic parameter indicates the susceptibility to minor measurement difficulties during the CPET and the number of outliers due to the determination method which were excluded.

Figure 2 presents violin plots of all analyzed kinetic parameters for NYHA class I, II and III and the age and sex-matched healthy reference group. In addition to the kinetic parameters, violin plots were presented for CPET markers known to have high predictive value (2) including $\dot{V}O_{2peak}$, OUES and $\dot{V}E/\dot{V}CO_2$ slope for comparison.

The z-scores (Table 2) show that *τ $\dot{V}O_2$ on-kinetics* was the best $\dot{V}O_2$ on-kinetic parameter to discriminate between healthy participants and patients with HF. *Slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* and *% rel $\dot{V}O_2$ reduction 60sec post test* performed best among the $\dot{V}O_2$ off-kinetic parameters. These three parameters were therefore considered superior to the others and further analyses were limited to these parameters.

Quantile curves for *τ $\dot{V}O_2$ on-kinetics, slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* and *% rel $\dot{V}O_2$ reduction 60sec post test* are presented in Figure 3. The quantile curves based on the healthy participants tend toward pathological numbers with increasing age. For the parameter *τ $\dot{V}O_2$ on-kinetics,* 60% of the HF patients were located above the 50th percentile. For the *slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* 85% of the HF patients were located below the 50th percentile, and for *rel $\dot{V}O_2$ reduction 60sec post test,* 78% were located above the 50th percentile.

Table 1: *Descriptive characteristics of the study population separated into healthy participants and patients with heart failure by NYHA functional classes.*

	Healthy		Healthy controls*		NYHA I		NYHA II		NYHA III	
Participants, no. (%)	N	526	N	158	N	37	N	28	N	14
Sex (m/f)		275/251		129/29		35/2		20/8		9/5
Age (yr)	526	54 ± 19.6	158	65.9 ± 13.7	37	65.4 ± 13	28	64 ± 14.3	14	72.9 ± 10.7
Height (cm)	526	171.6 ± 9.2	158	173.9 ± 9	37	174.8 ± 6.6	28	172.1 ± 8.3	14	168.4 ± 9.1
Body mass (kg)	526	69.9 ± 11.6	158	74.8 ± 11.8	37	85.9 ± 14.1	28	84.5 ± 16.4	14	78.4 ± 18.4
BMI (kg/m^2)	526	23.7 ± 2.7	158	24.7 ± 2.8	37	28.1 ± 4.0	28	28.3 ± 4.0	14	27.7 ± 6.6
Resting systolic BP (mmHg)	525	126.9 ± 13.9	158	131.6 ± 12.8	37	128 ± 13.7	28	127.8 ± 21.9	14	130.1 ± 15.1
Resting diastolic BP (mmHg)	525	77.4 ± 9	158	81.4 ± 7.8	37	79.4 ± 12.2	28	77.7 ± 14.4	14	75.9 ± 8.4
Etiology, ischemic, n (%)		n.a.		n.a.	37	28 (76)	28	21 (75)	14	11 (79)
Medication, n (%)										
Anti-Hypertensives (%)	526	46 (9)	158	25 (15)	37	36 (97)	28	25 (89)	14	14 (100)
of which ACE/ARB (%)	526	44 (8)	158	20 (13)	37	32 (86)	28	21 (75)	14	12 (86)
Beta-Blockers (%)	526	12 (2)	158	8 (5)	37	28 (76)	28	22 (79)	14	8 (57)
Anti-coagulants (%)	526	20 (4)	158	13 (8)	37	33 (89)	28	24 (86)	14	12 (86)
Statins (%)	526	22 (4)	158	14 (9)	37	31 (84)	28	20 (71)	14	11 (79)
Diuretics (%)	526	18 (3)	158	10 (6)	37	18 (48)	28	14 (50)	14	9 (64)
Anti-diabetics (%)	526	0 (0)	158	0 (0)	37	6 (16)	28	5 (17)	14	3 (21)
Blood testing										
HbA1c (mg/dL)	518	5.2 ± 0.4	157	5.4 ± 0.4	37	5.9 ± 0.7	27	6.0 ± 0.6	13	6.3 ± 0.7
LDL cholesterol (mg/dL)	518	122.6 ± 28.3	157	127.1 ± 26.1	37	89.6 ± 25.3	27	89.8 ± 18.1	13	94.4 ± 34.8
HDL cholesterol (mg/dL)	518	65.6 ± 14.9	157	62.8 ± 13.6	37	50.9 ± 9	27	53.3 ± 12.3	13	55.7 ± 11.7
Total cholesterol (mg/dL)	518	220.2 ± 42.3	157	227.2 ± 39.6	37	168.8 ± 41.3	27	172.4 ± 30.5	13	177.7 ± 54.4
Triglyceride (mg/dL)	518	117.4 ± 62.2	157	127.6 ± 54.4	37	140.1 ± 88.3	27	155.8 ± 109.4	13	103.5 ± 30.2
NTproBNP (pg/mL)	518	121.4 ± 209.6	157	108.3 ± 93.8	37	543.3 ± 573	27	580 ± 802.2	13	821.1 ± 655.5
Performance										
Pmax (W)	526	203.2 ± 84.0	158	200.3 ± 80.6	37	153.1 ± 44.0	28	116.1 ± 44.7	14	81.7 ± 30
VO_{2max} absolute ($L*min^{-1}$)	526	2.4 ± 0.8	158	2.4 ± 0.8	37	2 ± 0.5	28	1.7 ± 0.6	14	1.2 ± 0.3
VO_{2max} relative ($mL*kg^{-1}*min^{-1}$)	526	34.9 ± 10.3	158	32.5 ± 9.7	37	23.9 ± 5.9	28	19.9 ± 5.6	14	16.3 ± 4.6
RER_{max}	526	1.17 ± 0.08	158	1.14 ± 0.08	37	1.09 ± 0.08	28	1.06 ± 0.07	14	1.04 ± 0.08
HR_{max} (bpm)	507	169.9 ± 21.1	155	161.5 ± 21.8	37	136.6 ± 20.9	28	137.7 ± 26.5	14	127.2 ± 21.8
VO_2 reduction 60sec post test (L/min)	506	0.74 ± 0.37	154	0.69 ± 0.34	37	0.46 ± 0.25	28	0.42 ± 0.27	14	0.23 ± 0.19
VO_2 reduction 120sec post test (L/min)	504	1.19 ± 0.50	154	1.15 ± 0.47	37	0.93 ± 0.34	28	0.76 ± 0.41	14	0.48 ± 0.28

Abbreviations: BMI, body mass index; BP, blood pressure; HR, heart rate; Pmax, maximal power; VO_{2max}, maximal oxygen uptake; RER_{max}, maximal respiratory exchange ratio; HRmax, maximal heart rate.
*two participants from the healthy cohort were matched to every patient with heart failure according to age and sex (2:1 matching).
A description of the methods for the participants characteristics can be found in the supplement.

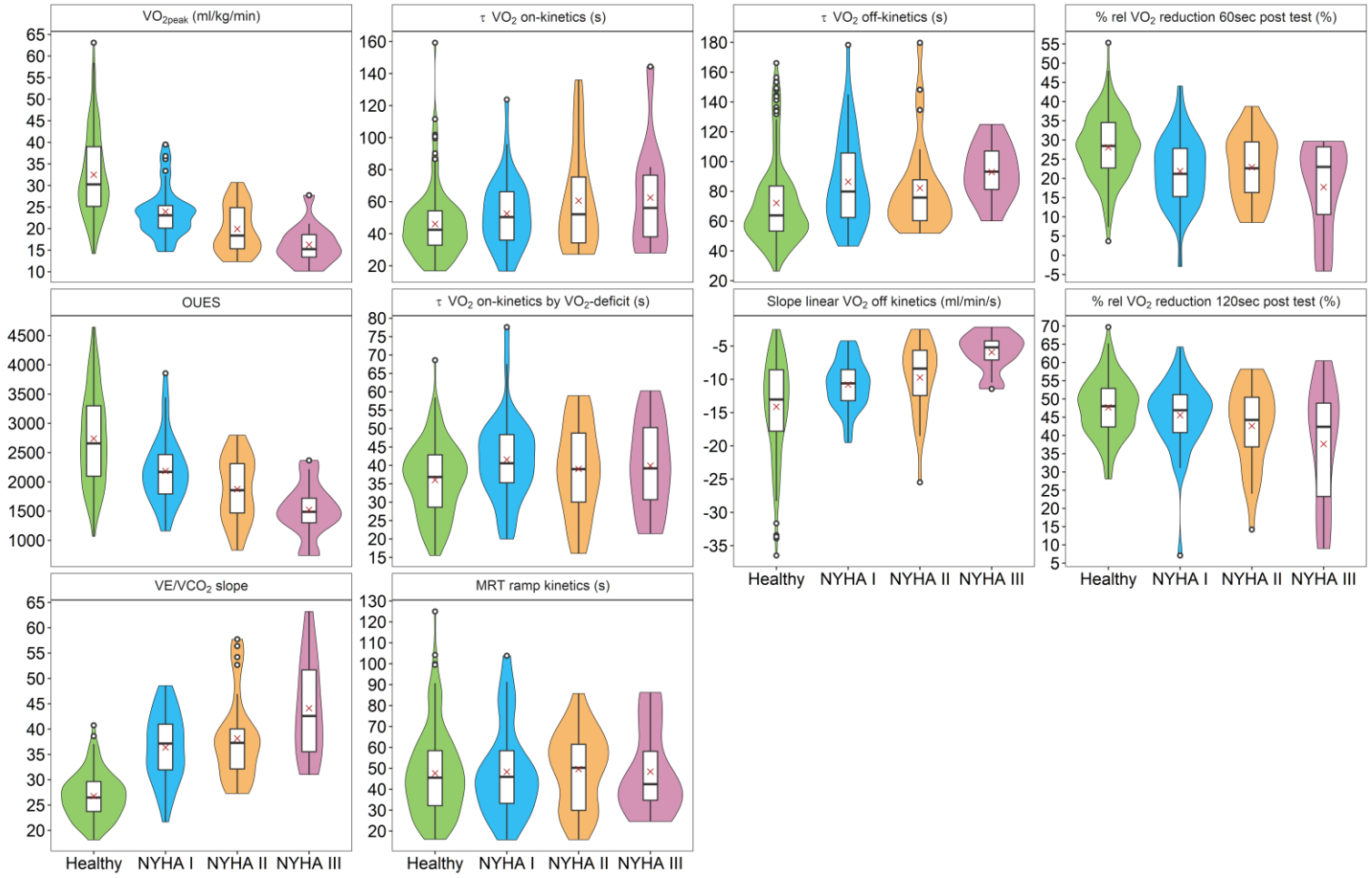

Figure 2: *Comparison of VO_2 on-kinetic und VO_2 off-kinetic parameters between a healhty control group and patients with heart failure with NYHA functional classes I, II and III.*

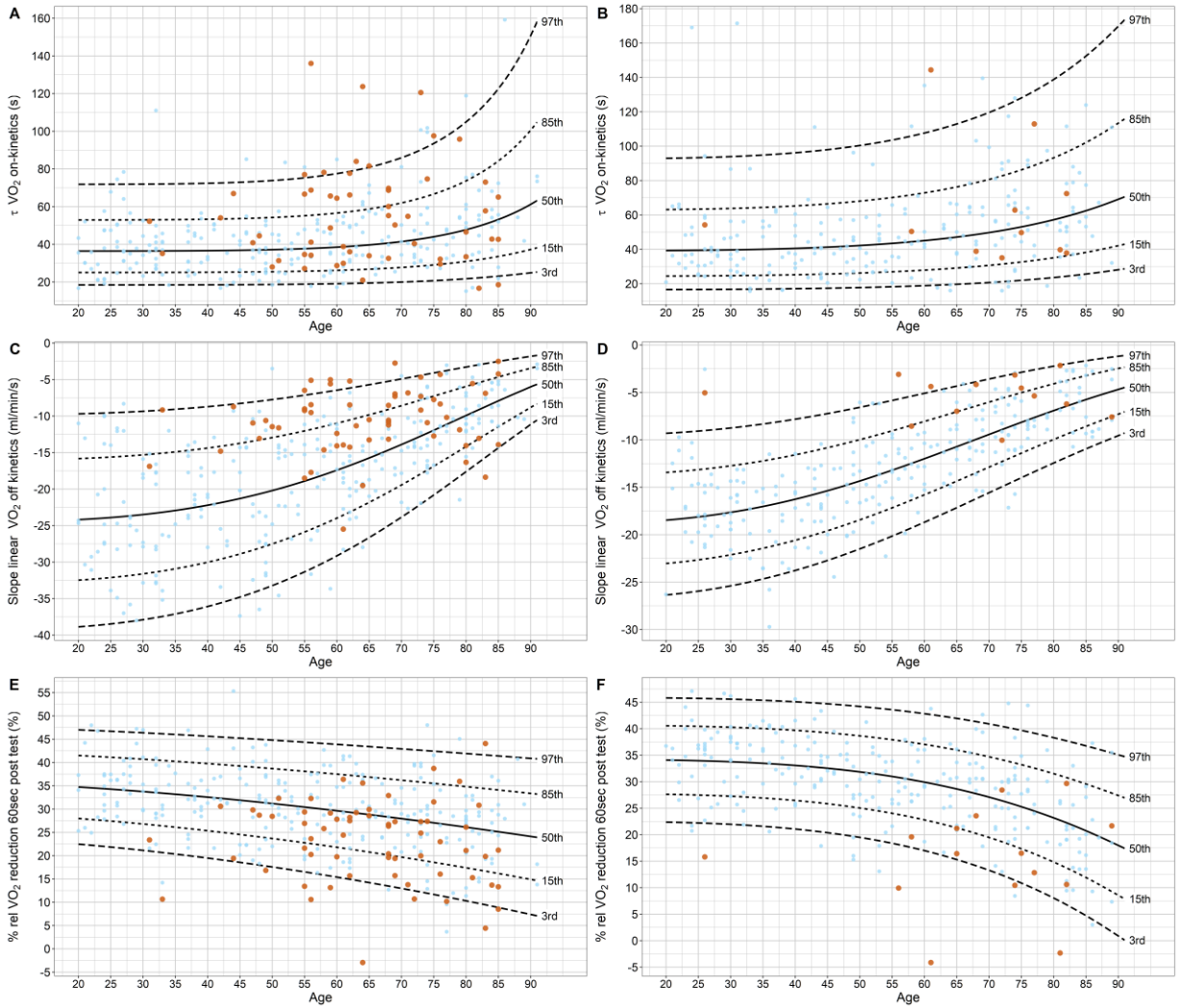

Figure 3: *Quantile curves for τ VO_2 on-kinetics, slope linear VO_2 off-kinetics (ml/min/s) and % rel VO_2 reduction 60sec post test for males and females, separately.*

Association of $\dot{V}O_2$-kinetics and $\dot{V}O_{2peak}$

There was strong evidence for associations between $\dot{V}O_{2peak}$ (mL/kg/min) and all $\dot{V}O_2$ on- and off-kinetics parameter except for *MRT ramp kinetics* in which there was no evidence for such an association observed (Table 2, last three columns). The direction of the association can be described as follows: the faster the $\dot{V}O_2$-kinetic response (depending on the parameter, a positive or negative association) the higher the $\dot{V}O_{2peak}$ values are observed. By far the largest adjusted R^2 was observed for the *slope linear $\dot{V}O_2$ off-kinetics (ml/min/s).*

Predicting disease severity (NYHA class) using kinetic parameters

Table 3 shows the results of several models for NYHA class prediction by kinetic parameters. All kinetic parameters improved the model when the base model contained sex and age. As indicated by the Chi^2, *slope linear $\dot{V}O2$ off-kinetics (ml/min/s)* improved the model from the three kinetic parameters most but not to the extent that $\dot{V}O_{2peak}$ did.

Additional value of kinetics to predict disease severity

There was little evidence that any of the three kinetic parameters improved the models already containing sex, age and $\dot{V}O_{2peak}$. The adequacy index comparing the base models containing age, sex and $\dot{V}O_{2peak}$ to a model additionally containing the kinetic parameters was between 0.98 and 0.99. This means that the base models without the $\dot{V}O_2$ kinetic parameters contain nearly all the predictive information already.

Table 2: *Group differences between healthy participants and patients with heart failure, Z-scores and the association with $\dot{V}O_{2peak}$ for all kinetic parameters.*

		Group differences & HF	Healthy	Z-scores			Association with V̇O2peak*		
Parameter	**N**	**mean difference (95%-CI)**	***p*-value**	**Healthy**	**HF**	**mean difference Healthy-HF (95% CI)**	**coefficient estimate (95% CI)**	**partial R^2**	***p*-value**
τ $\dot{V}O_2$ on-kinetics (s)	529	0.16 (0.04;0.28)	0.007	0.00	0.48	-0.48 (-0.79;-0.16)	-0.01 (-0.01;-0.002)	0.04	0.005
τ $\dot{V}O_2$ on-kinetics by $\dot{V}O_2$-deficit (s)	541	0.07 (-0.00;0.15)	0.055	0.00	0.43	-0.43 (-0.78;-0.09)	-0.01 (-0.01;-0.003)	0.04	0.000
MRT ramp kinetics (s)	514	0.00 (-0.13;0.12)	0.963	0.01	0.02	0.02 (-0.30;0.26)	-0.0003 (-0.01;0.0005)	0.00	0.899
τ $\dot{V}O_2$ off-kinetics (s)	558	0.17 (0.06;0.27)	0.001	0.00	0.74	-0.74 (-1.04;-0.43)	-0.011 (-0.015;-0.008)	0.09	<0.001
slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	567	4.96 (3.63;6.30)	0.000	-0.01	0.88	-0.88 (-1.15;-0.62)	-0.46 (-0.51;-0.41)	0.39	<0.001
% rel $\dot{V}O_2$ reduction 60sec post test (%)	582	-5.76 (-7.94;-3.59)	0.000	0.00	-0.89	0.89 (0.59;1.18)	0.37 (0.28;0.46)	0.16	<0.001
% rel $\dot{V}O_2$ reduction 120sec post test (%)	579	-3.54 (-5.84;-1.23)	0.003	0.00	-0.64	0.64 (0.29;0.98)	0.34 (0.25;0.44)	0.12	<0.001

Abbreviations: HF, heart failure; τ, tau; MRT, mean response time; $\dot{V}O_2$, oxygen uptake; rel, relative.

*including data of healthy participants and patients with heart failure.

Table 3. *Predicting disease severity (NYHA functional class) using $\dot{V}O_2$ kinetic parameters*

Base Model	Additional variable	Adequacy of base model	Likelihood ratio test
Sex, age	τ $\dot{V}O_2$ on-kinetics (s)	0.88	$\chi^2(1)$ = 7.87, *p* = 0.005
Sex, age	% rel $\dot{V}O_2$ reduction 60sec post test	0.54	$\chi^2(1)$ = 53.81, *p* < 0.001
Sex, age	slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	0.51	$\chi^2(1)$ = 56.15, *p* < 0.001
Sex, age	$\dot{V}O_{2peak}$	0.27	$\chi^2(1)$ = 176.82, *p* < 0.001
Additional value			
$\dot{V}O_{2peak}$, sex, age	τ $\dot{V}O_2$ on-kinetics (s)	0.98	$\chi^2(1)$ = 5.36, *p* = 0.02
$\dot{V}O2_{peak}$, sex, age	% rel $\dot{V}O_2$ reduction 60sec post test	0.99	$\chi^2(1)$ = 0.54, *p* = 0.46
$\dot{V}O_{2peak}$, sex, age	slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	0.99	$\chi^2(1)$ = 0.46, *p* = 0.50

Abbreviations: NYHA class, New York Heart Association-Classification; $\dot{V}O_2$, oxygen uptake; τ, tau; rel, relative

Discussion

To our knowledge, the current study is the first to provide detailed $\dot{V}O_2$ kinetic results in large cohorts of healthy participants and patients with HF. All previously suggested methods to calculate $\dot{V}O_2$ on- and off-kinetics for risk stratification using a standard ramp protocol were analyzed and compared. Our results show that the $\dot{V}O_2$ off-kinetics according to *rel $\dot{V}O_2$ reduction 60sec post test (%)* or *slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* present an alternative to evaluate aerobic function and disease severity if $\dot{V}O_{2peak}$ cannot be determined. Additional value beyond that of $\dot{V}O_{2peak}$ for risk stratification of HF patients was not provided by $\dot{V}O_2$ on- or off-kinetics.

Differences between healthy participants and patients with HF

This study provides evidence that $\dot{V}O_2$-kinetic parameters differ between healthy participants and a group of mild to moderate functionally impaired patients with HF for all kinetic calculation methods with the exception of *τ $\dot{V}O_2$ on-kinetics by $\dot{V}O2$-deficit* and *MRT of the ramp kinetics*. The observed differences are in line with previous findings showing that patients with HF had significantly slower $\dot{V}O_2$-kinetics compared to healthy volunteers (8-10, 13). The slowing of $\dot{V}O_2$-kinetics in HF is closely related to impaired ventricular-pulmonary vascular function (5, 6) and/or impaired peripheral oxygen utilisation (26). In contrast to previous findings, *MRT of the ramp kinetics* and *τ $\dot{V}O_2$ on-kinetics by $\dot{V}O2$-deficit* did not distinguish between healthy participants and patients with HF. This is potentially caused by low reliability of *MRT of the ramp kinetics* compared to other $\dot{V}O_2$-kinetic measurements (27) and a rather short constant load period, which was used to calculate $\dot{V}O_2$ on-kinetics. The latter may have led to an overestimation of $\dot{V}O_2$ on-kinetics (i.e. the time constant τ may be underestimated), especially in individuals with slow $\dot{V}O_2$-kinetics

because they did not achieve an entire steady-state in $\dot{V}O_2$ during the three-minute constant load warm-up period.

Mean differences in Z-scores (Table 2) clearly indicate that $\dot{V}O_2$ off-kinetics, irrespective of the calculation method discriminate better between healthy participants and patients with HF compared to $\dot{V}O_2$ on-kinetics. The likely reason behind this observation is the demand to fit a regression into the data points for the increase in $\dot{V}O_2$ from a resting state to a warm-up phase with work rates of only 7 (unloaded) to 50 watts, depending on the chosen exercise protocol. The increment seems to be too low and the approach therefore prone to outliers. Furthermore, the respiratory rate at the beginning of a rest to exercise transition is rather low (i.e. 10-20 breaths per minute). As a result, the determination of on-kinetic markers is based on only a small number of data points during the early phase of the transition. In contrast, the work rate difference during the off transition is much higher compared to the on transition. Additionally, at the beginning of the off-transition respiratory rate is equal to the rate at incremental test termination (i.e. 3-7 times higher than resting respiratory rate), which potentially allows for a more precise determination of $\dot{V}O_2$-kinetics when a common CPET is used.

These results are in line with previous research showing that off-kinetics can be determined with greater fidelity (6) and higher reproducibility (28) than on-kinetics in patients with HF. Further, irrespective of the methodological difficulties with on-kinetics, off-kinetics may discriminate patients with HF better from their healthy counterparts as has been observed in a previous study (8).

The comparison between the off-kinetics parameters (different calculation approaches) revealed a higher potential to distinguish healthy participants and patients with HF for *% rel $\dot{V}O_2$ reduction 60sec post test* and *slope linear $\dot{V}O_2$ off-kinetics* compared to *% rel $\dot{V}O_2$ reduction 120sec post test* and *τ $\dot{V}O_2$ off-kinetics*. Interestingly, both superior off-kinetics parameters were determined from the first minute of the recovery period only while the other parameters were calculated from the first two minutes (*% rel $\dot{V}O_2$ reduction 120sec post test)* or the entire recovery duration (*τ $\dot{V}O_2$ off-kinetics).* This indicates that the very early phase of the off transition better distinguished between healthy participants and patients with HF.

Association with $\dot{V}O_{2peak}$

Strong significant associations between $\dot{V}O_{2peak}$ and off-kinetics were observed. *Slope linear $\dot{V}O2$ off-kinetics* explained 39% of the variation in $\dot{V}O_{2peak}$ among healthy participants and patients with HF. In contrast, $\dot{V}O_2$ on-kinetics showed significant but only weak associations with $\dot{V}O_{2peak}$; the on-kinetics parameter τ $\dot{V}O2$ (s) explained only 4% of the variation in $\dot{V}O_{2peak}$. The stronger association of the off-kinetics compared to the on-kinetics can likely be explained by the methodological considerations of the on-kinetics described above.

A recent study showed that the level of exhaustion had no impact on $\dot{V}O_2$ off-kinetics (29). That the determination of $\dot{V}O_2$-kinetics, unlike $\dot{V}O_{2peak}$, does not require the subject to perform the test

to maximal voluntary exertion is a large advantage. Many patients lack the motivation to perform a maximal exercise test, are not familiarized with severe exercise or may have a contraindication to maximal exertion (30). In contrast, the successful determination of $\dot{V}O_{2peak}$ requires either a $\dot{V}O_2$-plateau or a confirmation of a secondary exhaustion criteria (3). Considering the large existing evidence base for the valuable information $\dot{V}O_2$-kinetics provides coupled with the present results, $\dot{V}O_2$ off-kinetics can be suggested as potential substitute for $\dot{V}O_{2peak}$.

Predicting disease severity

The ability of a model to predict health status and disease severity of the patients with HF improved significantly when the $\dot{V}O_2$ on-kinetic parameter (*τ $\dot{V}O_2$ on-kinetics*) and the $\dot{V}O_2$ off-kinetic parameter *(% rel $\dot{V}O_2$ reduction 60sec post test*) were added. However, only $\dot{V}O_2$ off-kinetics added substantial information to the model. Thus, $\dot{V}O_2$ off-kinetics could be a tool to discriminate not only between healthy participants and those with mild functional impairment (NYHA class I), but also between NYHA classes as visualized by Figure 2. Our results are in line with previous studies showing the potential of $\dot{V}O_2$-kinetics for risk stratification (12, 15) but are in contrast to others who did not demonstrate better predictive value by the addition of $\dot{V}O_2$ off-kinetics (9-11).

Since we could already show the association with $\dot{V}O_{2peak}$ - unofficially considered the gold standard criteria for risk stratification - another established parameter, NYHA functional classes, were used to stratify the risk of HF patients. Based on the different underlining physiological aspects represented by $\dot{V}O_{2peak}$ and $\dot{V}O_2$-kinetics (5, 8) some additional predictive value of $\dot{V}O_2$-kinetics could be expected. However, our results showed minimal evidence of additional value of $\dot{V}O_2$ on- or off-kinetics. Two reasons likely explain these results: i) $\dot{V}O_{2peak}$ is already a very strong risk predictor in HF patients and the association of $\dot{V}O_{2peak}$ and NYHA class is already known to be high; and ii) $\dot{V}O_2$-kinetics are likely to provide the same predictive information as $\dot{V}O_{2peak}$, which is underscored by the association between $\dot{V}O_2$ off-kinetics and $\dot{V}O_{2peak}$ in this study. Therefore, even though we observed that $\dot{V}O_2$ kinetics has predictive value, it does not appear to have value beyond $\dot{V}O_{2peak}$.

Practical applications

Our results indicate that the method of quantifying $\dot{V}O_2$-kinetics is critical to its clinical application. They suggest that the determination of $\dot{V}O_2$ on-kinetics from rest to a light constant load phase is not optimal; rather, the results favour the analysis of off-kinetics when using a ramp protocol. The calculation of *rel $\dot{V}O_2$ reduction 60sec post test (%)* or *slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* is recommended to distinguish between healthy individuals and patients with HF. Since $\dot{V}O_2$ off-kinetics is not affected by the level of exhaustion (29), these parameters can be used as a substitute for $\dot{V}O_{2peak}$ when maximal exhaustion is not reached or when $\dot{V}O_{2peak}$ cannot be interpreted.

Using some basic spreadsheet calculation tools, the calculation of *rel $\dot{V}O_2$ reduction 60sec post test (%)* and *slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)* are quite simple (see supplement). To facilitate the

routine application of $\dot{V}O_2$ off-kinetics in the clinical setting, we recommend that the incorporation of these parameters in CPET application software.

Limitations

Our study has several limitations. First, we studied patients with light to moderate HF, with only a few patients in NYHA class III. Second, the study was cross-sectional, and therefore no hard endpoints such as mortality or hospitalization were available. To further improve the reliability and validity of the $\dot{V}O_2$ on- and off-kinetics determination, a warm-up and a cool-down phase of five minutes instead of three minutes could be applied.

Conclusion

Differences in $\dot{V}O_2$-kinetics between healthy participants and patients with HF are observed and are highly dependent on how they are calculated. $\dot{V}O_2$ off-kinetics appears to be superior for distinguishing patients with HF and healthy participants compared to $\dot{V}O_2$ on-kinetics and ramp-kinetics. If $\dot{V}O_{2peak}$ can not be determined, $\dot{V}O_2$ off-kinetics provides an acceptable substitute. However, additional value beyond that of $\dot{V}O_{2peak}$ can not be provided by $\dot{V}O_2$-kinetics.

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Conflicts of interest: none declared

Author contributions

Conception and design of the research: J.W., M.N., J.M., R.K, D.I., A.S-T.
Acquisition of data: J.W., R.K.
Analysis and interpretation of the data: J.W., M.N., J.M., D.I., R.K., A.S-T.
Statistical analysis: DI
Obtaining funding and supervising the work: A.S.T, R.K.
Drafting the manuscript: J.W., M.N.
Critical revision of the manuscript for important intellectual content: D.I., J.M., O.P., A.S.-T., R.K.

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Chapter 8

Publication 6

Composite Measure of Physical Fitness Discriminates between Healthy Aging and Heart Failure: the COmPLETE Study.

Authors:

Jonathan Wagner[1], Raphael Knaier[1], Karsten Königstein[1], Christopher Klenk[1], Justin Carrard[1], Eric Lichtenstein[1], Hubert Scharnagl[2], Winfried März[2,3,4], Henner Hanssen[1], Timo Hinrichs[1], Arno Schmidt-Trucksäss[1]* and Konstantin Arbeev[5]*

*shared Senior Authorship

[1] Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland
[2] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
[3] Medical Clinic V, Medical Faculty Mannheim, University of Heidelberg, Germany
[4] SYNLAB Academy, SYNLAB Holding Deutschland GmbH, Mannheim, Germany
[5] Biodemography of Aging Research Unit (BARU), Social Science Research Institute, Duke University, Durham, NC, USA

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Abstract

Background

Aging and changing age demographics represent critical problems of our time. Physiological functions decline with age often ending in a systemic process that contributes to numerous impairments and age-related diseases including heart failure (HF). We aimed to analyze whether differences in composite measures of physiological function (health distance, HD), specifically physical fitness, between healthy individuals and patients with HF, can be observed.

Methods

The COmPLETE Project is a cross-sectional study of 526 healthy participants aged 20-91 years and 79 patients with stable HF. Fifty-nine biomarkers characterizing fitness (cardiovascular endurance, muscle strength and neuromuscular coordination) and general health were assessed. We computed HDs as the Mahalanobis distance for vectors of biomarkers (all and domain-specific subsets) that quantified deviations of individuals' biomarker profiles from "optimums" in the "reference population" (healthy participants aged <40 years). We fitted linear regressions with HD outcomes and disease status (HF/Healthy) and relevant covariates as predictors and logistic regressions for the disease outcome and sex, age, and age^2 as covariates in the base model and the same covariates plus combinations of one or two HDs.

Results

Nine out of ten calculated HDs showed evidence for group differences between Healthy and HF ($p \leq 0.002$) and most models presented a negative estimate of the interaction term age by group ($p < 0.05$ for eight HDs). The predictive performance of the base model for HF cases significantly increased by adding HD *General health* or HD *Fitness* (AUCs 0.63, 0.89 and 0.84, respectively). HD *Cardiovascular endurance* alone, reached an AUC of 0.88. Further, there is evidence that the combination of HDs *Cardiovascular endurance* and *General health* shows superior predictive power compared to single HDs.

Conclusion

HD composed of physical fitness biomarkers differed between healthy individuals and patients with HF, and differences between groups diminished with increasing age. HDs can successfully predict HF cases, and HD *Cardiovascular endurance* can significantly increase the predictive power beyond classic clinical biomarkers. Applications of HD could strengthen a comprehensive assessment of physical fitness and may present an optimal target for interventions to slow the decline of physical fitness with aging and, therefore, to increase healthspan.

Introduction

Aging and changing age demographics potentially represent one of the most critical problems of our time (1, 2). The shift of the major causes of morbidity towards chronic disease, coupled with changing age demographics, likely leads to an epidemic of age-driven chronic disease. Cardiovascular disease is the leading cause of death worldwide (3). It includes heart failure (HF) which is a complex multi system clinical syndrome. The prevalence of HF continues to rise in sync with the aging population (4). Physiological functions decline with age, and these declines often end in a systemic process that contributes to numerous physiological impairments and age-related diseases, including HF (5, 6). The ability to perform physical tasks is critical for maintaining overall functional capacity. Physical fitness is one domain of physiological functions declining with advancing age (7-9). Physical fitness measures are biomarkers of health, predicting quality of life, disability, and mortality (9-12). The inverse relationship between cardiovascular risk factors or cardiovascular disease and physical fitness markers such as cardiorespiratory fitness or hand grip strength have been widely described (13, 14). Physical fitness markers can be separated into three subdomains: cardiovascular endurance, muscle strength, and neuromuscular coordination (15). So far, a narrow focus on non-physical biomarkers in clinical assessments, however, persists. Further, when physical function is assessed, it is often performed only by a single parameter such as the measurement of grip strength, gait speed, or some measure of cardiorespiratory fitness. Physical fitness including all components has not been studied comprehensively so far (16).

Measurements of different physiological biomarkers, particularly physical fitness ones, provide an opportunity for personalized predictions of upcoming changes in an individual's health and onset of diseases and, eventually, death (17). Such biomarkers can manifest underlying age-related changes in physiological dysregulation that propagate to deteriorating health and result in increased risks of adverse outcomes with age. Both individual biomarkers and composite measures based on multiple biomarkers have been studied in relation to morbidity and mortality outcomes (see, e.g., recent reviews in (18, 19)). Recently, the statistical (Mahalanobis) distance (D_M; denoted in the context of this paper as health distance, HD), constructed based on the joint distribution of multiple biomarkers, was proposed as a composite measure that can represent the level of physiological dysregulation in an aging organism. It can be used as a measure of aging-related declines in robustness and resilience and as a preclinical indicator of an individual's transition from a healthy to an unhealthy state (20, 21). Numerous studies have confirmed the association of D_M with mortality, diseases, and aging-related outcomes (20-24), and there is emerging evidence on genetic determinants of the rates of physiological dysregulation represented by D_M (25). However, applications of this measure to broader sets of biomarkers, including physical fitness ones, and studies of their association with impaired health status such as HF are still lacking. In this paper, we constructed HD using, for the first time, biomarkers of physical fitness to test whether the levels of HD are associated with health status in the COmPLETE Project (16). Biomarkers of all physical fitness domains were included.

The aims of this study were: 1) to analyze whether differences in the composite measures (HD) between healthy individuals and patients with HF can be observed. 2) to describe how HD changes with increasing age in health and HF, 3) to compare domains of physical fitness summarized in multiple HDs against each other and against HD of standard clinical biomarkers and 4) to analyze whether HD can increase sensitivity and specificity in the discrimination process between healthy individuals and patients with HF.

Material and Methods

Population and recruitment

The COmPLETE Project is a cross-sectional single-center study performed between 2018 and 2019 in Basel, Switzerland. The project comprised two parts, COmPLETE-Health and COmPLETE-Heart. COmPLETE-Health included 526 healthy men and women aged 20–91 years equally distributed across age decades and sex. The participants have a body mass index <30 kg/m^2, and are nonsmokers or ex-smokers for more than 10 years. Exclusion criteria included any kind of exercise–limiting chronic disease and blood pressure > 160/100 mmHg. COmPLETE-Heart included 79 cardiac patients with stable HF with NYHA functional classes I-III; thus, symptoms and signs have remained unchanged for at least one month. Diagnosis of HF was confirmed on clinical history, physical examination, assessment of N-terminal pro Brain Natriuretic Peptide (NT-poBNP) and echocardiographically demonstrated relevant structural heart disease or diastolic dysfunction according to the European Society of Cardiology guidelines (26). The exact recruitment procedure and the full list of inclusion and exclusion criteria can be found in the study protocol (16).

Setting

This study was carried out at the Department of Sport, Exercise, and Health at the University of Basel, Switzerland, and was funded by the Swiss National Science Foundation (grant no. 182815). It was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451). Written informed consent was obtained from all study participants prior to inclusion.

Data

General health domain

Height and body mass were measured to the nearest 0.5 cm and 0.1 kg, respectively, and the body mass index was calculated. A four-segment bioelectrical impedance analysis was conducted (Inbody 720; Inbody Co. Ltd., Seoul, South Korea) to measure percentage body fat, lean body mass and skeletal muscle mass. Resting systolic and diastolic blood pressures, resting heart rate, pre-ejection period, ejection time of the left ventricle, brachial-ankle pulse wave velocity (baPWV), and cardio–ankle vascular index (CAVI) were measured with the participant in the supine position using a non-invasive vascular screening system (VaSera VS-1500 N; Fukuda Denshi, Tokyo, Japan). Smoking status was assessed by telephone interview prior to the appointment, whereas physicians reviewed medical history and medications by onsite questionnaires. Forced vital

capacity (FVC) and forced expiratory volume in one second ($FEV_{1)}$, objective parameters of respiratory function, were measured in accordance with the American Thoracic Society/European Respiratory Society guidelines (27) immediately before the exercise test (28). Blood samples were drawn via venipuncture by trained medical staff in fasting status (at least three hours, mean five hours). Blood samples were immediately centrifuged, and the plasma aliquots were frozen at −80 °C. Cholesterol, triglycerides and high- (HDL) and low- (LDL) density lipoprotein concentrations were measured from serum using enzymatic reagents (DiaSys, Holzheim, Germany) and were calibrated using secondary standards (Roche Diagnostics, Mannheim, Germany). High-sensitive C-reactive protein was measured using a particle enhanced immunoturbidimetric assay (Diasys, Holzheim, Germany). Measurements were performed on an Olympus AU680 automatic analyzer (Beckman Coulter, Brea, CA, USA). HbA1c was quantified from whole blood by high pressure liquid chromatography using D-10 (Bio-Rad, Hercules, CA, USA). NT-proBNP was determined using a chemiluminescent microparticle immunoassay (Architect, Abott, IL, USA). All tests were performed according to the manufacturer's recommendations.

Cardiovascular Endurance domain

A cardiopulmonary exercise test (CPET) until maximal exertion was performed using an electromagnetically braked cycle ergometer (Ergoselect 200; Ergoline, Bitz, Germany) and applying a ramp protocol. Gas exchange and ventilatory variables were analyzed continuously (breath-by-breath) using a computer-based system (MetaMax 3B; Cortex Biophysik GmbH, Leipzig, Germany). All tests were continued until maximal exertion (i.e., volitional exertion, dyspnea, or fatigue). Before and during the test, patients were encouraged to reach their level of maximal exhaustion. Peak oxygen uptake (peak $\dot{V}O_{2)}$ was defined as the highest 30-second average of $\dot{V}O_2$ at any point during the test. $\dot{V}O_2$ off-kinetics were assessed from the active recovery period that directly followed the incremental phase of the CPET. A complete description of the CPET can be found elsewhere (29).

Muscle strength and power domain

Isometric lower body strength was measured performing a mid-thigh pull using an analogue dynamometer (TTM Muscular Meter, Tokyo, Japan). Countermovement jumps (CMJ) were performed on a force plate (Leonardo Mechanograph®, Novotec Medical, Pforzheim, Germany) to measure peak power and jump height. Maximal strength and rate of force development (RFD) of the handgrip were measured on the dominant side using a handheld dynamometer (Leonardo Mechanograph GF; Novotec Medical GmBH, Pforzheim, Germany).

Neuromuscular coordination domain

Balance was assessed by the path length of the center of pressure during an upright static tandem stance using the same force plate as for the CMJ. Gait parameters were assessed during habitual walking speed on a 20-m walkway using an inertial sensor system (Physilog®; GaitUp, Lausanne, Switzerland).

Physical Activity domain

Physical Activity (PA) was objectively measured continuously over 14 days using a wrist-worn triaxial accelerometer (GeneActive Activinsights Ltd., Kimbolton, UK). The device was attached to the participant's nondominant wrist and sampled data at a frequency of 50 Hz. The numbers of minutes per day performed at light (1.5–3.99 METS; metabolic equivalent of task), moderate (4.00–6.99 METS), and vigorous (≥ 7 METS) PA were averaged for all valid days (30).

The exact sequence and detailed description of methods of the various measurements beyond the explanations above are described elsewhere (16).

Statistical analysis

Participant characteristics were analyzed descriptively. The distribution of continuous variables was inspected graphically and characterized by means and standard deviations. Categorical variables are presented as absolute and relative frequencies. *P*-values ≤ 0.05 were considered statistically significant.

Analyses of Health Distance

The Health Distance (HD) is the composite measure constructed from a set of biomarkers as recently suggested (31). It is also known as the measure of physiological dysregulation (32). This is the Mahalanobis distance (33, 34) defined for vectors of biomarker measurements and it quantifies the deviations of individuals' biomarker profiles from "optimal" (or "reference") values in a "reference population." This "reference population" can be represented by a subsample from the same study or some other sample can be used for this purpose. For a (column) vector of biomarkers measured in an individual *i*, x_i, the health distance HD_i is defined as $HD_i = \sqrt{(x_i - \bar{x})^T S^{-1} (x_i - \bar{x})}$, where $\bar{x}$ is a vector of means and *S* is the variance-covariance matrix of the respective biomarkers calculated in the "reference" population (superscript *T* denotes transposition).

In this study, we constructed different variants of HDs based on the subset of biomarkers available in the COmPLETE Study. The initial list of 59 biomarkers is shown in Table 1. We excluded four biomarkers from the initial list since they were included in the in- or exclusion criteria (BMI, rest systolic and diastolic BP and NT-proBNP). In addition, the following biomarkers were excluded due to their high correlation to other biomarkers (absolute values of pairwise correlations exceeding 0.9): height, lean body mass, FEV1, LDL cholesterol, peak $\dot{V}O_2$ (mL/kg lean mass/min), peak workload, and peak $\dot{V}E$. The selection of one variable within correlated groups of variables was based on previous evidence and guidelines (35, 36). Selected parameters are, therefore, more likely to be associated with aging, general health outcomes, or HF.

The resulting list of 48 biomarkers was included in the "All Biomarkers HD" and we also computed HDs from domain-specific sets of biomarkers indicated in Table 1 (Anthropometry; Vascular and respiratory health; Blood testing; Cardiovascular endurance; Muscle strength/power;

Neuromuscular coordination; and Physical activity). In addition, we used respective Cardiovascular endurance, Muscle strength, Neuromuscular coordination, and Physical activity biomarkers to construct the "Fitness biomarkers HD," and Anthropometry, Blood, and Vascular and respiratory health biomarkers to compute the "General health" HD.

As there were missing values in biomarkers (see Table 1), we performed multiple imputation of missing values of biomarkers using the R-package "mice" (37). We generated 25 datasets with imputed values of biomarkers and computed HDs in each dataset using the observed and imputed values as described below (see also section *Sensitivity Analyses* regarding different imputation methods).

Prior to computations of HDs, biomarker values were transformed using the Box-Cox transformation and standardized to be on the same scale (mean = 0 and variance = 1). For biomarkers with negative values, the observations were shifted by adding a constant so that the values would be in the positive range. For computations of HDs, we selected healthy individuals younger than 40 years as the "reference population." This cutoff resulted in a reasonably large reference population and a sizable healthy group (see also section *Sensitivity Analyses* regarding different definitions of the reference population). In each imputed dataset, we computed the means and the variance-covariance matrix in this "reference population" separately for females and males and used them in constructing HDs from observed and imputed values of biomarkers for each individual of respective sex as in the above formula. The original HDs were then transformed using the Box-Cox transformation and standardized to a zero mean and a unit variance. Note that the original HDs are positive numbers by construction (see the formula above) whereas the Box-Cox transformed ones have negative values. Thus, zero values of HDs in respective figures can be viewed as the average values of the HDs in the sample.

For each computed HD, we fitted the linear regression model with HD as the dependent variable and the disease status (0 – healthy, 1 – HF), sex (0 – male, 1 - female), age_40 (computed as age – 40), age_40^2, smoking status (0 – never smoked, 1 – ever smoked), medication use (0 – do not use, 1 – use medications indicated in Table 1), and the interaction term for the disease status and age_40 as independent variables (see also section *Sensitivity Analyses* regarding different specifications of the regression model). The output from the analyses in each imputed dataset was pooled using the standard Rubin's rules. The pooled estimates were used to compute the estimated values of HDs from the respective regression equation in each stratum of the dichotomous variables and for ages in the range from 40 to 91. The age trajectories of HDs for healthy and HF corresponding to the "female non-smokers not taking medications" stratum are reported in respective figures.

We also fitted the logistic regression model for the disease status as the outcome and sex, age_40 and age_40^2 as covariates in the base (reference) model and the same covariates plus combinations of one or two HDs (HD1 only, HD2 only, HD1 and HD2; for specific types of HD1 and

HD2, see Results) to compare the performance of different models in predictions of HF cases. Neuromuscular coordination was not included in the AUC analysis due to the non-significant HD difference between Healthy and HF. We evaluated the areas under the receiver operating characteristic curves (AUC) and differences between those, along with values of sensitivities and specificities, in each imputed dataset. Leave-one-out cross-validation was used for model evaluation in each respective calculation. We reported median values and interquartile ranges of AUCs across all imputed datasets and estimated differences in AUC pooled using the standard Rubin's rules. The receiver operating characteristic (ROC) curves in each imputed dataset and the ROC curve drawn at median values of 1 – specificity and sensitivity across all imputed datasets are presented in the main text and in the Supplement.

Sensitivity Analyses

We performed sensitivity analyses to check sensitivity of results to various aspects of computational workflows which could hypothetically affect the estimates and conclusions. First, we used different specifications of imputation models for biomarkers in the multiple imputation procedure: a) age, sex, disease status; b) age, sex; c) age, sex, disease status, biomarkers; d) age, sex, biomarkers; e) age, sex, disease status, biomarkers, other covariates (such as smoking, medications); d) age, sex, biomarkers, other covariates. All results were similar in all imputation methods. Therefore, we report only the results for option a) in the paper. Second, we checked another cutoff to define the reference population (younger than 50 years). We replicated all results using the same models but with age_50 (computed as age - 50) and all conclusions were identical. Hence, only the baseline scenario with the cutoff age 40 is reported. Third, we checked other sets of covariates in the linear regression model (excluding smoking, medication, age_40^2, and the interaction term for the disease status and age_40). All estimates for the disease status variable were qualitatively similar in all models. We also tested the model with the interaction term for disease status and age_40^2 and the results for the disease status were similar to the model without this term. As the regression coefficient for the interaction term for the disease status and age_40^2 was not significant, we reported the model without this term.

Descriptive analyses, construction of HDs, linear regression analyses and tabulation of results were performed in R version 3.6.1 or later and in MATLAB R2019b. Logistic regression analyses were done in SAS 9.4 (SAS/STAT 14.3). MATLAB R2019b was used for visualization.

Results

Participant characteristics

A total of 526 healthy participants and 79 cardiac patients with HF (NYHA functional classes I – III) were included. All HF patients were in a stable condition; their etiology was cardiomyopathy (n=8), coronary artery disease (n=60), pulmonary hypertension (n=1), valvular regurgitation (n=8) or valvular stenosis (n=2). Subjects' characteristics and biomarkers are presented in Table 1 stratified by subgroups including young healthy individuals ≤ 39 years of age (Reference Population), healthy individuals ≥ 40 years of age (Healthy), and patients with HF (Heart Failure). The table indicates that participants from the HF group are approximately 2.3 years older on average compared to participants in the Healthy group ($p = 0.16$). The proportion of females in the both the Reference Population and the Healthy group are nearly 50%, whereas the proportion of females in the HF group is 19%. Medication use differs between the Reference Population, the Healthy group and the HF group and is most prevalent in the HF group. Differences described above are significant ($p < 0.0001$).

Table 1 also presents descriptive statistics for the biomarkers selected for computations of HD. Most of the biomarkers included in the HD calculation were highly correlated with age ($p < 0.0001$) (supplementary material, Table S1). The mean values of biomarkers differed between the reference population (≤ 39 years of age) and the Healthy group (≥ 40 years of age) in most biomarkers ($p < 0.0001$) (supplementary material, Table S2). We note however, that these results are purely descriptive and do not explore how multiple factors (except age) may contribute to such differences.

Health distances in health and heart failure

Group differences between Healthy individuals ≥ 40 years of age and patients with HF are reported in Table 2. Nine out of ten HD showed evidence for a difference between the groups ($p \leq 0.002$).HD trajectories for *Fitness* for the Healthy and HF group are presented from 40 to 91 years in Figure 1. Trajectories of additional HD can be found in the supplement (Figures S1-S9). The HD trajectories of the healthy group continuously increase starting with negative values to values of > 1.3 (see section Analyses of Health Distance regarding interpretation of zero HD). In contrast, the HF group HD trajectories already begin at a positive HD of approximately 0.4 and increase to a similar region as the healthy counterparts' HD reaching values of > 1.4 with 91 years. The largest HD difference between those groups is observed at the youngest age (40 years) after that the HD trajectories of *Fitness* of the Healthy and HF group continuously approach each other with increasing age. The approaching pattern of HD is observed in most of the calculated HDs presented in Table 2 (see Figures S1-S9).

Table 1. *Descriptive characteristics of the study population separated into Reference Population (healthy participants aged ≤ 40 years), Healthy (healthy participants aged ≥ 40 years) and Heart Failure (patients with heart failure). Data are presented as mean ± standard deviation if not stated otherwise.*

Characteristics	N	Reference Population	N	Healthy	N	Heart Failure	N	Total Sample	Included in HD
Participants, n (%)		152 (25.1)		374 (61.8)		79 (13.1)		605 (100.0)	n/a
Sex (m/f), n (%)	152	83 (54.6)/69 (45.4)	374	190 (50.8)/184 (49.2)	79	64 (81.0)/15 (19.0)	605	337 (55.7)/268 (44.3)	n/a
Age (yr)	152	29.6 ± 5.3	374	63.9 ± 13.7	79	66.2 ± 13.3	605	55.6 ± 19.3	n/a
NYHA class, n (%)									
I	152	0 (0.0)	374	0 (0.0)	79	37 (46.8)	605	37 (6.1)	n/a
II	152	0 (0.0)	374	0 (0.0)	79	28 (35.4)	605	28 (4.6)	n/a
III	152	0 (0.0)	374	0 (0.0)	79	14 (17.7)	605	14 (2.3)	n/a
Smoking status, n (%)									
Smokers	152	0 (0.0)	374	0 (0.0)	72	7 (8.9)	598	7 (1.2)	n/a
Never Smoked	152	142 (93.4)	374	280 (74.9)	72	38 (48.1)	598	460 (76.0)	n/a
Ex-Smokers >10 yr	152	10 (6.6)	374	94 (25.1)	72	27 (34.2)	598	131 (21.7)	n/a
Medication use, n (%)									
Medication	152	0 (0.0)	374	77 (20.6)	79	75 (94.9)	605	152 (25.1)	n/a
Antihypertensives	152	0 (0.0)	374	56 (15.0)	79	75 (94.9)	605	131 (21.7)	n/a
ACE ARB	152	0 (0.0)	374	44 (11.8)	79	65 (82.3)	605	109 (18.0)	n/a
Beta-blockers	152	0 (0.0)	374	12 (3.2)	79	58 (73.4)	605	70 (11.6)	n/a
Anticoagulants	152	0 (0.0)	374	20 (5.3)	79	69 (87.3)	605	89 (14.7)	n/a
Statins	152	0 (0.0)	374	22 (5.9)	79	62 (78.5)	605	84 (13.9)	n/a
Antidiabetics	152	0 (0.0)	374	0 (0.0)	79	14 (17.7)	605	14 (2.3)	n/a
Anthropometry									
Height (cm)	152	174.5 ± 8.5	374	170.4 ± 9.2	79	172.7 ± 8.0	605	171.7 ± 9.0	0
Body mass (kg)	152	70.2 ± 11.9	374	69.8 ± 11.5	79	84.1 ± 15.8	605	71.7 ± 13.1	1
BMI (kg/m2)	152	23.0 ± 2.7	374	23.9 ± 2.7	79	28.1 ± 4.5	605	24.2 ± 3.4	0
WHR	152	0.8 ± 0.1	374	0.9 ± 0.1	79	1.0 ± 0.1	605	0.9 ± 0.1	1
Body fat (%)	151	19 ± 7	369	25 ± 8	79	32 ± 7	599	24 ± 8	1
Lean body mass (kg)	151	56.7 ± 11.0	369	52.3 ± 10.2	79	57.2 ± 10.1	599	54.1 ± 10.6	0
Skeletal muscle mass (kg)	151	31.9 ± 6.8	369	28.8 ± 6.2	79	31.6 ± 6.1	599	30.0 ± 6.5	1
Vascular and respiratory health									
Rest systolic BP (mmHg)	152	121 ± 12	374	130 ± 13	79	127 ± 15	605	127 ± 14	0
Rest diastolic BP (mmHg)	152	72 ± 8	374	79 ± 8	79	78 ± 12	605	77 ± 9	0
HR at rest (bpm)	152	61 ± 10	372	61 ± 9	79	63 ± 13	603	62 ± 10	1
baPWV (m/s)	152	10.2 ± 1.0	374	13.3 ± 2.7	79	13.5 ± 2.7	605	12.6 ± 2.7	1
CAVI	152	6.2 ± 0.9	374	8.8 ± 1.5	79	9.2 ± 1.6	605	8.2 ± 1.8	1
Preejection period (ms)	151	98.9 ± 17.5	373	105.8 ± 17.0	77	121.6 ± 27.3	601	106.1 ± 19.9	1
Ejection time LV (ms)	152	307.6 ± 16.3	374	313.1 ± 22.1	79	304.3 ± 34.9	605	310.6 ± 23.2	1
FVC	139	5.0 ± 1.1	346	3.9 ± 1.0	62	3.6 ± 1.0	547	4.1 ± 1.2	1
FEV1	139	4.0 ± 0.8	346	3.0 ± 0.8	62	2.8 ± 0.7	547	3.2 ± 0.9	0
Blood testing									
NTproBNP (pg/ml)	145	79.2 ± 79.1	371	138.0 ± 240.6	77	603.1 ± 673.5	593	184.0 ± 350.4	0
HbA1c (mg/dL)	147	5.0 ± 0.3	371	5.3 ± 0.4	77	6.0 ± 0.7	595	5.3 ± 0.5	1
Total cholesterol (mg/dL)	147	191 ± 35	371	232 ± 39	77	172 ± 40	595	214 ± 45	1

Triglyceride (mg/dL)	147	113 ± 69	371	119 ± 59	77	139 ± 91	595	120 ± 67	1
HDL cholesterol (mg/dL)	147	62.0 ± 15.0	371	67.1 ± 14.6	77	52.6 ± 10.7	595	63.9 ± 15.1	1
LDL cholesterol (mg/dL)	147	102.6 ± 21.6	371	130.6 ± 26.7	77	90.5 ± 24.7	595	118.5 ± 29.9	0
C-reactive protein (mg/L)	147	1.60 ± 4.17	371	1.93 ± 3.31	77	3.65 ± 5.91	595	2.07 ± 3.99	1
Creatinine (mg/dl)	147	0.83 ± 0.14	371	0.84 ± 0.17	77	1.08 ± 0.43	595	0.87 ± 0.23	1
Cardiovascular endurance									
Peak $\dot{V}O_2$ (L/min)	152	3.00 ± 0.73	374	2.22 ± 0.78	79	1.77 ± 0.58	605	2.36 ± 0.85	1
Peak $\dot{V}O_2$ (mL/kg/min)	152	42.9 ± 7.9	374	31.7 ± 9.3	79	21.1 ± 6.2	605	33.2 ± 10.9	1
Peak $\dot{V}O_2$ (mL/kg leanmass/min)	151	52.9 ± 7.4	369	41.8 ± 9.5	79	30.7 ± 7.5	599	43.1 ± 11.1	0
Peak O_2pulse (mL/beat)	147	16.6 ± 3.6	362	14.1 ± 4.1	79	14.2 ± 4.2	588	14.7 ± 4.2	1
Peak workload (W)	152	257 ± 69	374	182 ± 80	79	127 ± 50	605	194 ± 84	0
$\dot{V}O_2$ at VT1 (mL/kg/min)	152	24.8 ± 6.0	374	19.5 ± 5.7	78	13.1 ± 4.1	604	20.0 ± 6.6	1
$\dot{V}O_2$ at VT1 (L/min)	152	1.74 ± 0.47	374	1.37 ± 0.47	78	1.09 ± 0.37	604	1.42 ± 0.50	1
$PETCO_2$ at rest (mmHg)	152	32.6 ± 2.8	374	31.0 ± 3.0	79	29.3 ± 3.0	605	31.2 ± 3.1	1
$PETCO_2$ at VT1 (mmHg)	152	43.7 ± 3.9	374	39.7 ± 4.1	78	35.6 ± 3.6	604	40.2 ± 4.7	1
$\dot{V}E/\dot{V}CO_2$ slope	152	34.5 ± 5.6	374	37.3 ± 6.6	79	41.9 ± 8.3	605	37.2 ± 6.9	1
$\dot{V}E/\dot{V}CO_2$ slope below VT2	152	26.8 ± 4.0	315	30.5 ± 5.1	79	37.8 ± 8.0	546	30.5 ± 6.3	1
OUES (mL/min)	152	3031 ± 719	374	2376 ± 799	79	1983 ± 650	605	2489 ± 833	1
OUES (mL/min/kg)	152	43.3 ± 8.9	374	33.9 ± 9.8	79	23.4 ± 6.6	605	34.9 ± 11.0	1
% rel $\dot{V}O_2$ reduction 60sec post test	139	33.9 ± 6.5	364	28.3 ± 8.2	78	21.6 ± 9.4	581	28.8 ± 8.8	1
slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	137	-19.9 ± 7.2	365	-13.4 ± 6.8	78	-9.0 ± 5.0	580	-14.3 ± 7.5	1
Peak Lac (mmol/L)	127	10.3 ± 2.2	328	7.0 ± 2.5	72	4.5 ± 1.6	527	7.4 ± 2.9	1
Peak $\dot{V}E$ (l/min)	152	128 ± 33	374	98 ± 35	79	82 ± 30	605	103 ± 37	0
Peak HR (bpm)	146	188 ± 9	361	162 ± 20	79	135 ± 23	586	165 ± 24	1
HRR 1 min (bpm)	142	-25 ± 9	356	-24 ± 10	77	-21 ± 9	575	-24 ± 10	1
HRR 2 min (bpm)	143	-59 ± 16	353	-52 ± 16	77	-48 ± 19	573	-53 ± 16	1
Peak exercise systolic BP (mmHg)	131	181 ± 22	320	191 ± 24	79	170 ± 31	530	185 ± 26	1
Muscle strength / power									
CMJ peak power (kN)	151	2.9 ± 0.8	362	2.1 ± 0.7	77	2.2 ± 0.8	590	2.3 ± 0.8	1
CMJ height (m)	151	0.26 ± 0.06	362	0.16 ± 0.06	77	0.13 ± 0.06	590	0.18 ± 0.08	1
Hand grip strength (N)	150	412.7 ± 115.5	364	344.0 ± 102.2	76	367.1 ± 97.7	590	364.5 ± 109.0	1
Hand grip RFD (N/150ms)	150	280.9 ± 83.0	364	226.8 ± 85.2	76	189.0 ± 65.1	590	235.7 ± 87.2	1
Isometric leg strength (kg)	152	135 ± 40	373	104 ± 40	76	106 ± 38	601	112 ± 42	1
Neuromuscular coordination									
COP path length (cm)	150	23.9 ± 8.0	357	42.0 ± 23.6	74	47.8 ± 20.2	581	38.1 ± 22.0	1
Gait speed (m/s)	145	1.4 ± 0.1	351	1.4 ± 0.2	78	1.3 ± 0.2	574	1.4 ± 0.2	1
Gait cadence (steps/minute)	145	113 ± 8	351	115 ± 8	78	112 ± 9	574	114 ± 8	1
Stride Length (m)	145	1.50 ± 0.11	351	1.46 ± 0.14	78	1.38 ± 0.15	574	1.46 ± 0.14	1
Gait double support (%)	145	21.9 ± 2.7	351	21.5 ± 3.0	78	22.3 ± 3.5	574	21.7 ± 3.0	1
Gait asymmetry (%)	145	2.2 ± 2.1	351	2.6 ± 2.9	78	2.7 ± 2.8	574	2.5 ± 2.7	1
Physical activity									
Light physical activity (min/day)	148	94 ± 26	358	103 ± 32	73	90 ± 36	579	99 ± 31	1
Moderate physical activity (min/day)	148	178 ± 50	358	157 ± 62	73	108 ± 60	579	156 ± 62	1
Vigorous physical activity (min/day)	147	9 ± 10	355	6 ± 9	73	2 ± 4	575	6 ± 9	1

Abbreviations: HD, health distance; n/a, not applicable; NYHA class, New York Heart Association functional classification; ACE, angiotensin-converting-enzyme; ARB, AT1-receptor-blocker; BMI, body mass index; WHR, waste-to-hip ratio; BP, blood pressure; HR, heart rate; baPWV, brachial-ankle pulse wave velocity; CAVI, cardio–ankle vascular index; LV, left ventricular; FVC, forced vital capacity, FEV1, forced expiratory volume in 1 second; LDL, low-density lipoprotein; HDL, high density lipoprotein; VO_{2max}, maximal oxygen uptake; VT, ventilatory threshold; $P_{ET}CO_2$ = partial pressure of end-tidal CO_2; VCO_2, carbon dioxide output; VE, volume of expiration; OUES, oxygen uptake efficiency slope; Lac, lactate; HRR, heart rate recovery; CMJ, counter movement jump; RFD, rate of force development, COP, center of pressure

Table 2. *Estimates of group differences in Health Distance for Healthy (healthy participants aged ≥ 40 years) and Heart Failure (patients with heart failure). Estimates and standard errors are for age 40 years.*

Health Distance	estimate	SE	*p*-value	*adjusted p*-value
All biomarkers	1.05	0.15	< 0.0001	< 0.0001
Fitness biomarkers	0.95	0.17	< 0.0001	< 0.0001
Cardiovascular endurance biomarkers	1.44	0.18	< 0.0001	< 0.0001
Muscles strength biomarkers	1.36	0.21	< 0.0001	< 0.0001
Neuromuscular coordination biomarkers	0.26	0.26	0.3047	1.0000
Physical activity biomarkers	1.07	0.29	0.0002	0.0020
General health biomarkers	1.48	0.17	< 0.0001	< 0.0001
Anthropotmetry biomarkers	1.49	0.27	< 0.0001	< 0.0001
Blood biomarkers	1.66	0.27	< 0.0001	< 0.0001
Vascular and respiratory health biomarkers	1.15	0.16	< 0.0001	< 0.0001

Legend: Column "adjusted p-value" reports p-values adjusted for multiple comparisons using the Bonferroni correction

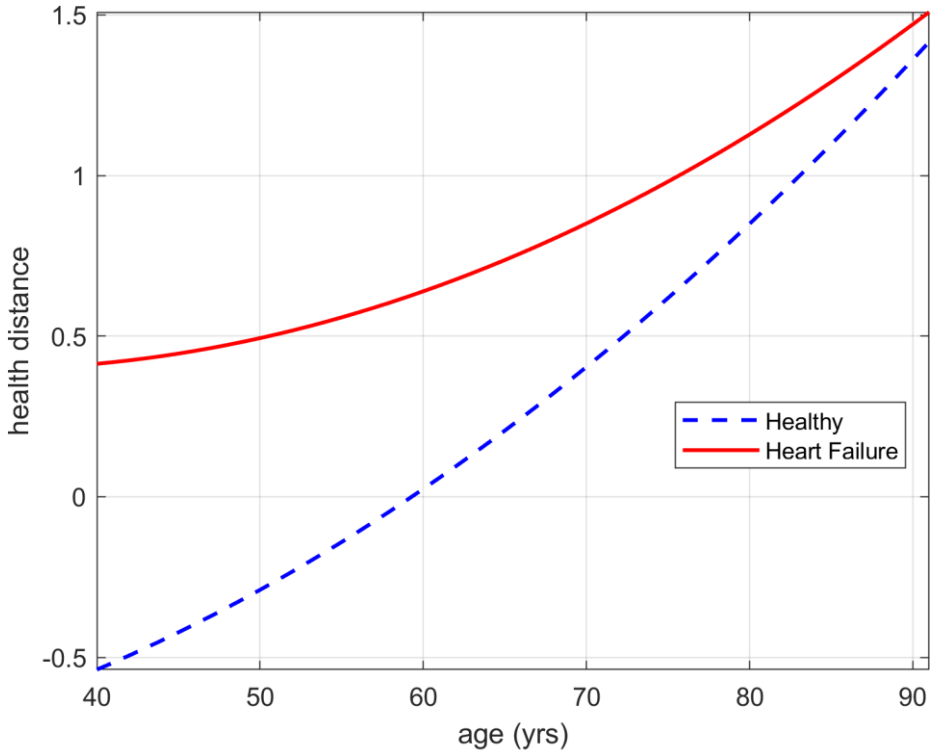

Figure 1: *Health distance trajectories for Fitness biomarkers for the Healthy and Heart Failure group presented from 40 to 91 years of age. The curves correspond to non-smoking females not taking medications.*

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Predicting HF cases using health distances

Discriminative performance analyses presented in Table 3 compare the performance of different models in predictions of HF cases (cardiac disease) for the total sample. The table shows the median estimates of the areas under the receiver operating characteristic curves (AUC) for three different models including different HDs and always including the base model (age, age^2, and sex). Further differences between AUC within these models are presented. Figure 2 indicates that compared to the base model with sex and age, both the *General health* and the *Fitness* biomarkers increase the AUC estimate significantly from 0.63 to 0.89 and 0.84, respectively. In addition, there is a significant additional benefit when combining these two health distances compared to one HD alone. Compared to the initial model with sex, age and age^2, both *Cardiovascular endurance* and *Muscle strength* biomarkers increase the AUC estimates substantially from 0.64 to 0.88 and 0.78, respectively (see also Figure 3). There is, however, little evidence that adding HD *Muscle strength* to HD *Cardiovascular endurance* adds value. Table 3 (see also Figure 4) shows that *Cardiovascular endurance* alone reaches an AUC of 0.88 compared to *General health* HD with 0.89. Further, there is evidence that the combination of both HD *Cardiovascular endurance* and HD *General health* shows superior predictive power compared to one of the HDs alone.

Table 3. *Areas under receiver operating characteristics curves (AUC) in models with different health distances (HD) as predictors of Heart Failure. AUC presented as median (IQR) across all imputed data sets.*

	AUC	AUC difference			
Model	**Median (IQR)**	**Contrast**	**Mean (SE)**	**95% CI**	***p*-value**
no HD	0.632				
General health HD (GH HD)	0.886 (0.010)	GH HD - no HD	0.252 (0.033)	[0.189, 0.316]	< 0.0001
Fitness HD (F HD)	0.838 (0.013)	F HD - no HD	0.202 (0.032)	[0.139, 0.266]	< 0.0001
General health HD and Fitness HD	0.908 (0.006)	GH HD and F HD - no HD	0.275 (0.032)	[0.213, 0.338]	< 0.0001
		GH HD and F HD – GH HD	0.023 (0.010)	[0.004, 0.042]	0.0200
		GH HD and F HD - F HD	0.073 (0.021)	[0.032, 0.114]	0.0005
		GH HD – F HD	0.050 (0.028)	[-0.004, 0.104]	0.0718
no HD	0.632				
Cardiovascular endurance HD (CVE HD)	0.877 (0.006)	CVE HD - no HD	0.246 (0.033)	[0.182, 0.311]	< 0.0001
Muscle strength HD (MS HD)	0.780 (0.005)	MS HD - no HD	0.147 (0.031)	[0.086, 0.207]	< 0.0001
Cardiovascular endurance HD and Muscle strength HD	0.889 (0.005)	CVE HD and MS HD - no HD	0.257 (0.033)	[0.192, 0.323]	< 0.0001
		CVE HD and MS HD – CVE HD	0.011 (0.008)	[-0.005, 0.027]	0.1694
		CVE HD and MS HD – MS HD	0.111 (0.021)	[0.069, 0.153]	< 0.0001
		CVE HD – MS HD	0.100 (0.026)	[0.049, 0.151]	0.0001
no HD	0.632				
General health HD (GH HD)	0.886 (0.010)	GH HD - no HD	0.252 (0.033)	[0.189, 0.316]	< 0.0001
Cardiovascular endurance HD (CVE HD)	0.877 (0.006)	CVE HD - no HD	0.246 (0.033)	[0.182, 0.311]	< 0.0001
General health HD and Cardiovascular endurance HD	0.927 (0.003)	GH HD and CVE HD - no HD	0.294 (0.032)	[0.232, 0.356]	< 0.0001
		GH HD and CVE HD - GH HD	0.042 (0.012)	[0.017, 0.066]	0.0009
		GH HD and CVE HD - CVE HD	0.048 (0.015)	[0.018, 0.077]	0.0015
		GH HD – CVE HD	0.006 (0.024)	[-0.042, 0.054]	0.8041

Abbreviations: HD, health distance; GH, general health; F, fitness; CVE, cardiovascular endurance; MS, muscle strength; IQR, interquartile range; SE, standard error; CI, confidence interval

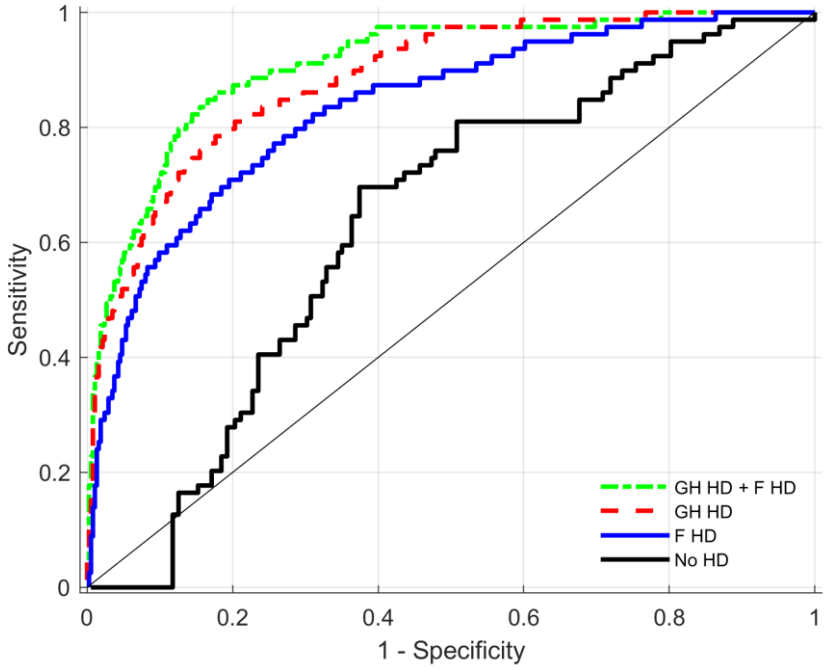

***Figure 2**: Receiver operating characteristics (ROC) curves for Health distances (HD) of General health (GH), Fitness (F) and the combination of both HDs as predictors of Heart Failure. ROC curves present combined output from all imputed data sets (see Material and Methods).*

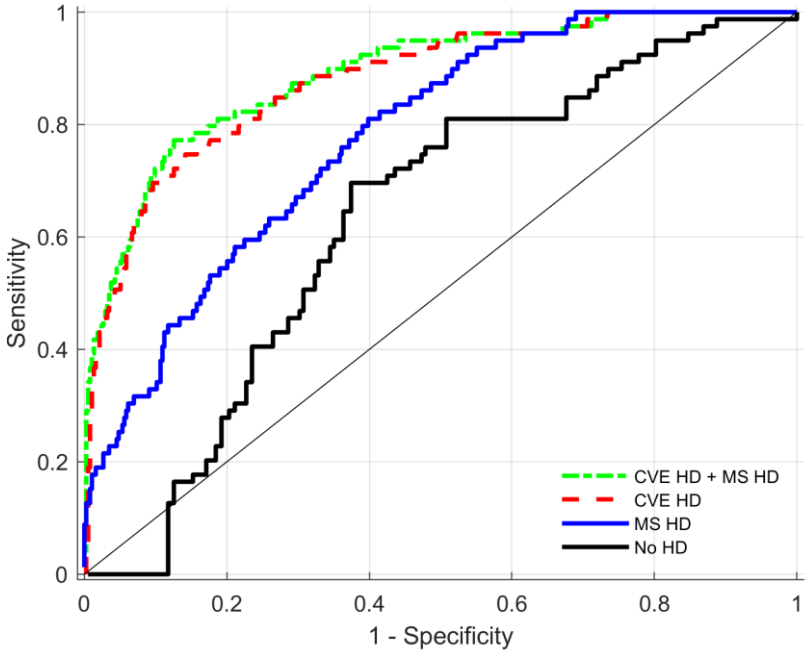

***Figure 3:** Receiver operating characteristics (ROC) curves for Health distances (HD) of Cardiovascular endurance (CVE), Muscle strength (MS) and the combination of both HDs as predictors of Heart Failure. ROC curves present combined output from all imputed data sets (see Material and Methods).*

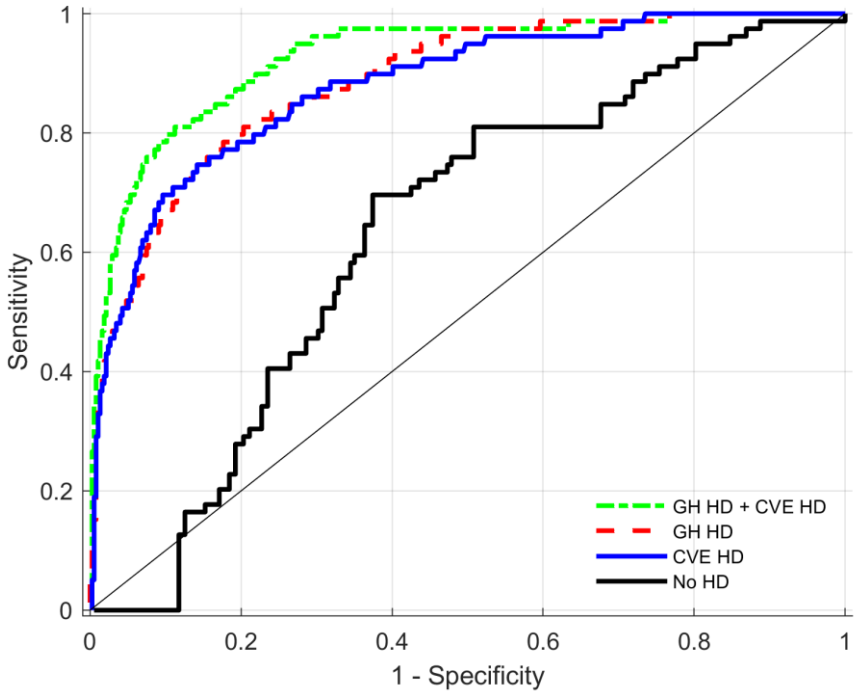

Figure 4: *Receiver operating characteristics (ROC) curves for Health distances (HD) of General health (GH), Cardiovascular endurance (CVE) and the combination of both HDs as predictors of Heart Failure. ROC curves present combined output from all imputed data sets (see Material and Methods).*

Discussion

Our study is the first to comprehensively measure all physical fitness components in both a healthy sample over the life span from 20–91 years and in patients with HF. Further, the study applied a novel approach from the field of epidemiology and research on aging to physiological biomarkers of physical function. Our results showed that HD composed of physical fitness or standard clinical health biomarkers differ between healthy individuals and patients with HF and that these differences reduce with increasing age. Further, HD of physical fitness can significantly increase the predictive power to detect HF cases in our sample beyond sex and age but also beyond classic clinical biomarkers.

Differences between healthy participants and patients with HF

Age- and sex-adjusted differences between healthy individuals and patients with HF can be observed in various combinations of biomarkers summarized to composite outcomes (HD). Both HD *Fitness* and HD *General health* show evidence for a difference between groups. Out of the fitness domains, cardiovascular endurance and muscle strength but not neuromuscular performance differed between groups. The observed differences in the composite outcome

Cardiovascular endurance and *Muscle strength* do support previous findings showing that single markers of the strength domain such as isometric leg strength or handgrip strength (38-40) and markers of cardiovascular endurance such as peak $\dot{V}O_2$, OUES, or $\dot{V}E/\dot{V}CO_2$ are reduced in patients with HF. Combining and summing several relevant biomarkers showing differences between these two groups already for themselves to one composite outcome unsurprisingly led to highly significant group differences in the present study because presumable true signals are summed. In contrast, our results did not show evidence for the composite outcome *Neuromuscular coordination* between healthy individuals and patients with HF. In the field of cardiology, measures of frailty have, however, gained attention in addition to strength and endurance performance tests (41). HF affects predominantly older individuals (42), and patients with HF have a higher prevalence of frailty (41, 43, 44). The assessment of gait speed has been demonstrated to be a reliable single marker of frailty in older patients with HF and gait speed is independently associated with death, hospitalization for HF, all-cause hospitalization and improves risk stratification (45). The HD *Neuromuscular coordination* composed of gait parameters and a balance measure may not provide relevant information in our sample because the mean age of the HF group was relatively young (66 years) and measures of neuromuscular coordination are deteriorated more commonly at older age (46).

Although closely related, PA deserves a distinct glance to the other fitness outcomes because it is a behavioral measure. In line with previous evidence examining PA patterns, PA behavior seems to differ in patients with HF compared to healthy individuals (47) and get worse with increasing clinical severity of HF (48). The known inverse association between fitness measures and the development of HF, and the potential to increase those fitness outcomes in patients with HF by PA, provides clinicians with a powerful tool. As observed in our study, HD *Fitness* and HD *PA* are both affected by HF and often present a vicious cycle between the behavioral component and the functional outcomes.

From the *General health* HDs, particularly HD *Blood markers* and HD *Anthropometry* observed large differences between the groups. Even though only unspecific blood markers were included (because NT-proBNP was excluded due to the utilization as HF group inclusion criteria) substantial group differences were observed and were largely age independent over the lifespan from 40–91 years.

Trajectories

Both groups (Healthy and HF) showed a curvilinear increase in HDs, with the largest difference observed at the youngest age of approximately 40 years. Both Healthy and HF seem to converge towards an unknown upper limit which might indicate disability or mortality. The decline of physiological functions with advancing age including physical fitness seems to be an unstoppable process and affects these functions whether a diagnosed chronic disease is present or absent. The trend toward a highly similar HD at the highest ages might be explained by the burden to take part

in such a study and by the decision of an individual or of the referring physician to enroll the patient. The upper limit of the described HDs for healthy elderly and HF might, therefore, describe the minimum level of physiological function and fitness required to be able to keep an appointment of several hours. In addition, the development of HF at an older age is probably characterized by better physical function and a better risk factor profile earlier in life, which might reduce HD between healthy individuals and patients with HF at old ages. According to our results and models, the older the individuals are, the more difficult it is to differentiate between early stages of HF and the biological effect of aging. Therefore, it can be argued that targeting physiological functions (fitness and general health functions) with increasing age is essential whether a manifest chronic disease (in our case HF) is present or absent. HD and, therefore, physiological dysregulation increases sharply with advancing age and, thus, likely decreases robustness and resilience of an individual.

Predicting HF cases

The fact that HD *Fitness* could detect HF patients with an estimate of AUC = 0.84 is a notable result and supports recent findings describing the importance of physical fitness assessment in clinical practice in general but specifically in patients with HF (14). Noteworthy is the finding that HD *Cardiovascular endurance* showed a higher AUC compared to HD *Fitness,* which includes the same biomarkers as *Cardiovascular endurance* but in addition the biomarkers of the fitness domains *Muscle strength* and *Neuromuscular function*. This finding indicates that including a larger number of biomarkers does not automatically improve the effect size of a composite measure consisting of physiological biomarkers. Including less but “relevant” biomarkers for the given task was superior in our data set. Summing “relevant” biomarkers improves the effect size whereas the inclusion of less “relevant” biomarkers worsens the effects size due to adding “noise” and diluting the signal. Similar observations were made in the field of genetics where application of the concept of “polygenic risk scores”, which combine effects of different genetic markers in one combined score, often result in similar findings (49).

The reason that HD *Muscle strength* does not add additional value to HD *Cardiovascular endurance* could be explained by noting that biomarkers included in HD *Cardiovascular endurance* such as peak $\dot{V}O_2$ (l/min) or peak lactate are likely associated with biomarkers of HD *Muscle strength* in our heterogeneous sample. Further, biomarkers included in HD *Cardiovascular endurance* might not only represent central but also peripheral limitations and muscle strength and power to some extent.

The observation that the combination of both HD *Cardiovascular endurance* and HD *General health* has superior discriminative performance than HD *General health* alone further strengthens the importance of cardiopulmonary exercise testing.

The described calculation approach of HD provides an interesting tool for future investigation and might have potential to discriminate healthy aging from the early beginning of chronic disease. It

could indicate when an overall accelerated decline beyond that typically observed in healthy aging begins and, therefore, mark the optimal starting point for specific exercise interventions to prevent age-related chronic diseases. Which biomarkers and which combinations of biomarkers should be included in an optimal HD measure requires further research.

Further, HD based on multiple biomarkers represent conceptually different components of the vulnerability to age-related disease compared to values of individual biomarkers, as argued by Arbeev et al. (21). HDs based on deviations of multiple biomarkers from their baseline states characterize the level of systemic dysregulation in physiological functions, which does not specifically require an individual biomarker to be highly abnormal or present a value typically seen in an individual with a chronic disease. For the composite measure HD, each deviation from the reference population may in principle lie within a clinically normal range; hence, the quantity and variety of biomarkers can contribute more to this composite estimate than the manifestation of any individual marker in regard to HF pathology. HD seems, particularly useful when the overall deviation of physiological functions, such as overall physical fitness, is of interest, independently of a specific disease. When a syndrome such as HF is multifaceted and impacts physical fitness over a variety of pathways, whereby both, central limitations (of the heart itself) and peripheral limitations within the skeletal musculature contribute to the overall reduction in physical functions (50), this approach can also be promising. HDs could be less specific than a single measure such as NT-proBNP, used for diagnosis of HF, but provide an overall measure of systemic dysregulation and reduction in physical fitness and thus an indication for a therapeutic approach.

Currently, there is a lack of physical fitness measurements in clinical practice as clinical vital signs and if assessed health care professionals pay attention to a single biomarker such as peak $\dot{V}O_2$. For this biomarker widely known cut-offs exist that correspond to clinical or preclinical manifestation of a particular disease. (e.g., a peak $\dot{V}O_2$ < 20 mL/kg/min is an indication of a mild to moderate impairment of HF according to the Weber classification (51)). HD, however, presents a more sophisticated approach using a cluster of abnormal values of different fitness biomarkers that occur together. The use of HD measures represents an additional step forward because it allows to utilize not only clinically “abnormal” values of physical fitness markers, but also those deviations from the baseline physiological state that, individually would not be considered as a clinically relevant reduction in physical fitness, but together may significantly contribute to the transition from healthy to unhealthy state.

Overall, this study demonstrates that a novel statistical tool, previously applied successfully in large-scale epidemiological studies using simpler biomarkers, can also be applied to physiological markers of physical function. This approach could further strengthen a comprehensive physical fitness assessment. It may help to find intervention and treatment options to decrease the accelerated decline of physiological function and, in particular, physical fitness accompanied with chronic disease and with the process of aging and thereby increase health span.

Limitations

Our study has limitations. First, this research was cross sectional and, therefore, no hard endpoints such as mortality or hospitalization were available. Second, the HF patient sample was rather small for investigating trajectories of HD over the age span, and the studied patients presented mostly light to moderate HF, with only a few patients with NYHA class III.

Conclusion

HD composed of physical fitness biomarkers differ between healthy individuals and patients with HF and those differences between groups diminish with increasing age. In both healthy individuals and patients with HF, HD tends towards a common unknown upper limit indicating frailty, disability or mortality. HDs can successfully predict HF cases, and HD *Cardiovascular endurance* can significantly increase the predictive power beyond classic clinical biomarkers. This study shows that a novel statistical tool from the field of epidemiology can be successfully applied to physiological biomarkers of physical function. The application of HD could strengthen a comprehensive physical fitness assessment and may present an optimal target for interventions to slow the decline of physical fitness with aging and, therefore, increase healthspan and delay the onset of chronic disease.

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451) and complied with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Consent for publication: not applicable

Availability of data and material: The datasets used for the current study are available from the corresponding author on request.

Competing interests: The authors declare that they have no competing interests.

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Author contributions:

Conceptualization, J.W., R.K., H.H., T.H., A.S.T and K.A.
Methodology, J.W., R.K., E.L., T.H., A.S.T and K.A.
Statistical analysis, K.A.
Investigation, J.W., R.K., K.K., C.K., J.C., H.S. and W.M.
Resources, A.S.T.

Data curation, J.W. and R.K.

Writing—original draft, J.W and K.A.

Writing—review and editing, R.K., K.K., C.K., J.C., E.L., H.S., W.M., H.H., T.H., D.S., A.S.T.

Project administration, J.W.

Funding acquisition, A.S.T.

All authors have read and approved the final manuscript.

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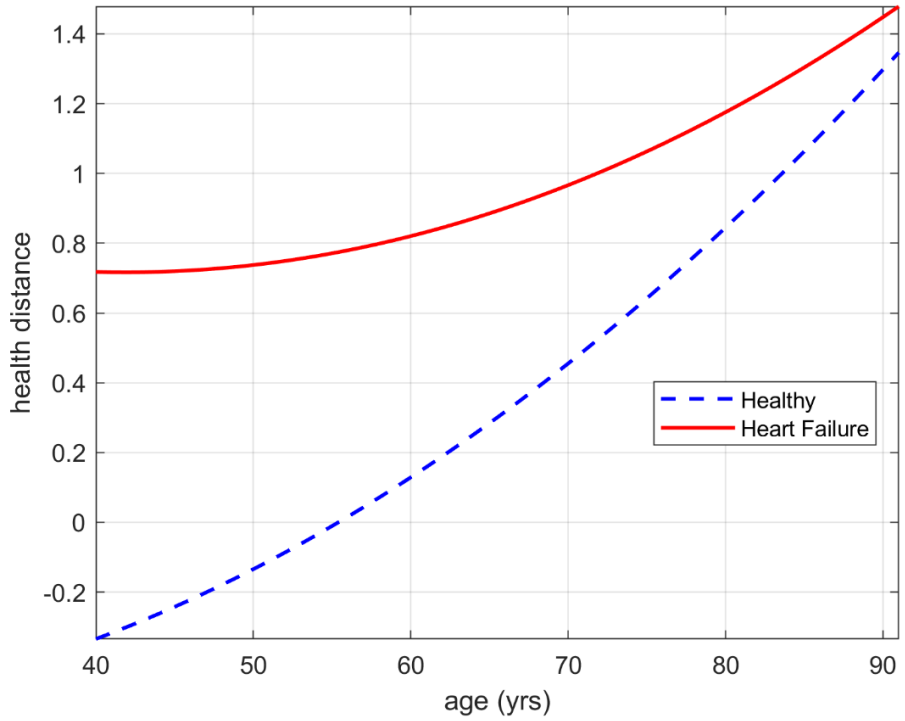
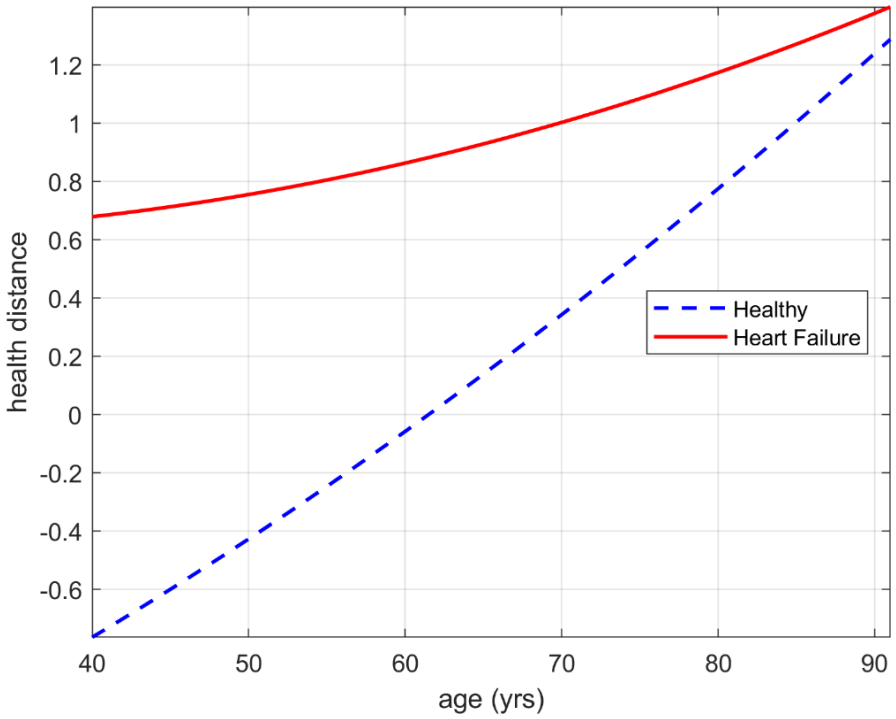
Supplementary material

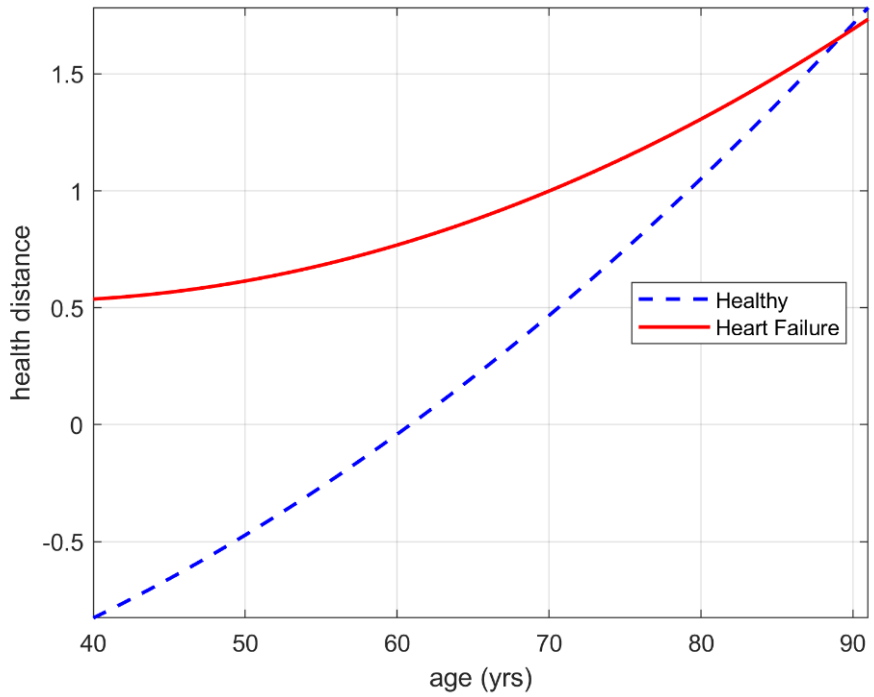
Supplementary Figures



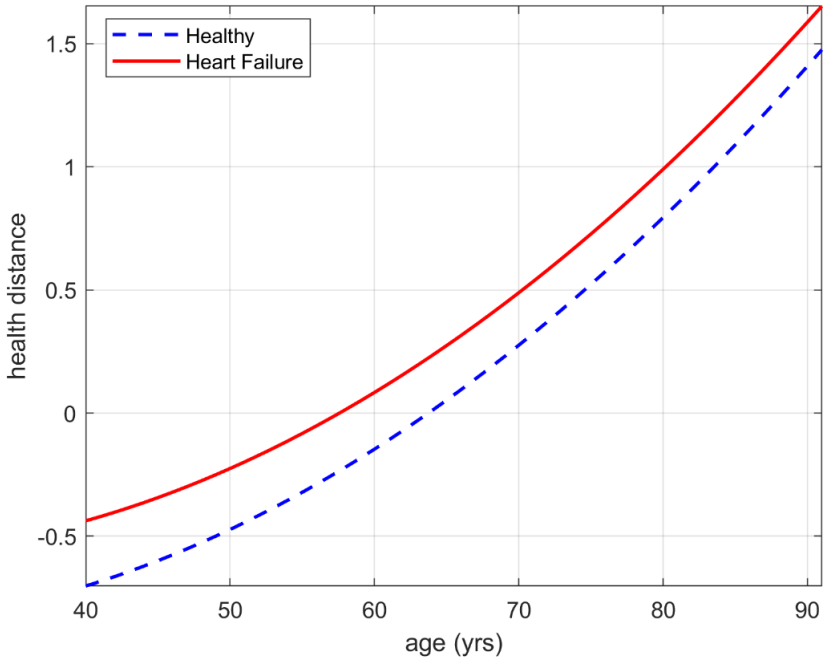
Supplementary Figure S1. Health distance trajectories for all biomarkers for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



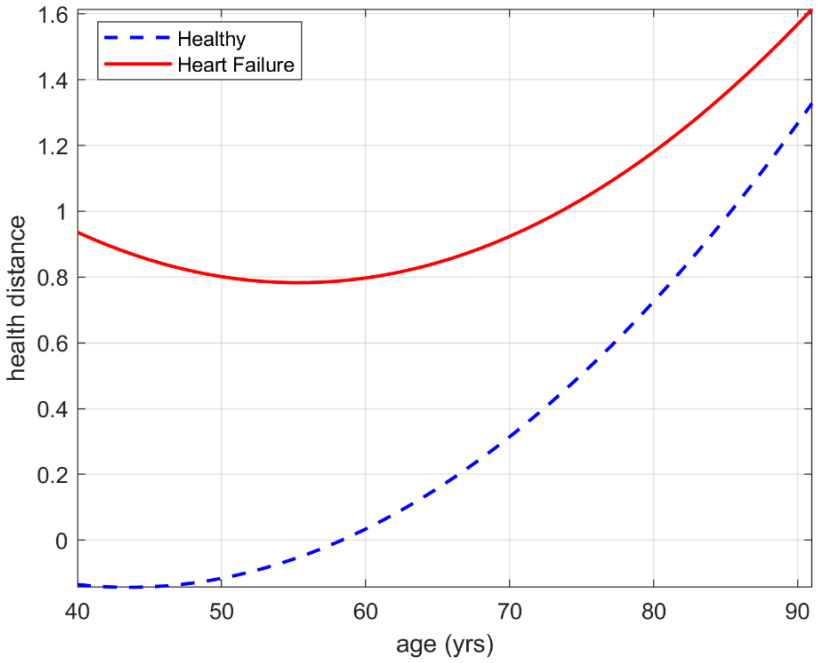
Supplementary Figure S2. Health distance trajectories for Cardiovascular endurance for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



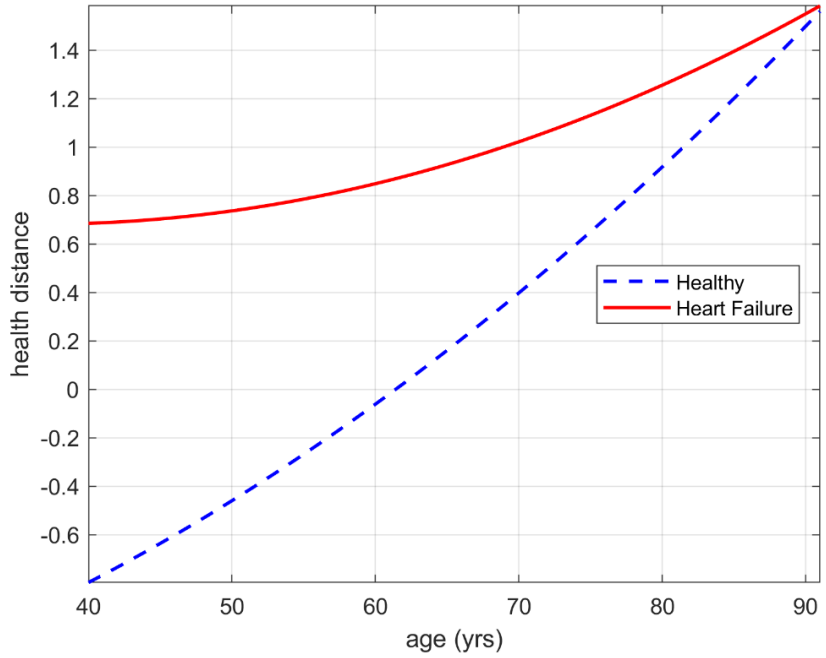
Supplementary Figure S3. Health distance trajectories for Muscle strength for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



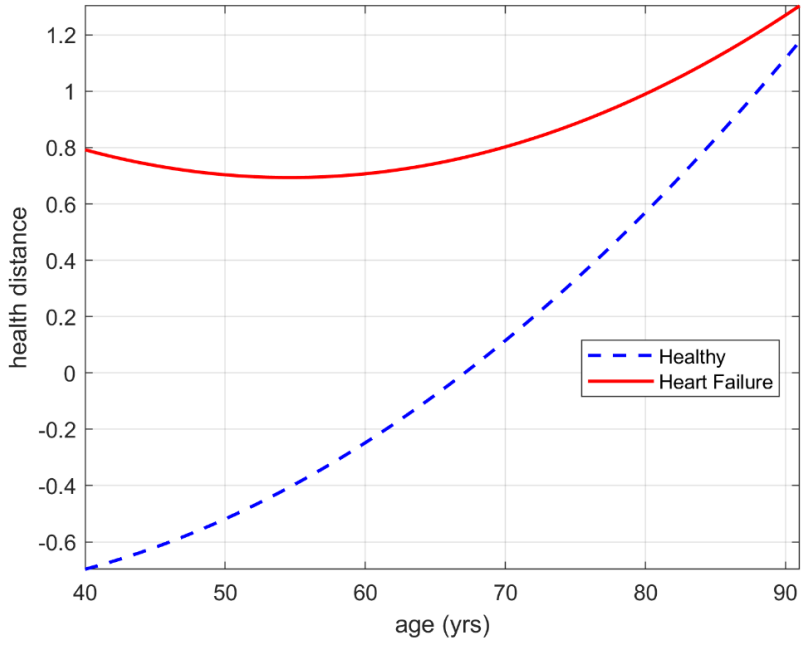
Supplementary Figure S4. Health distance trajectories for Neuromuscular coordination for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



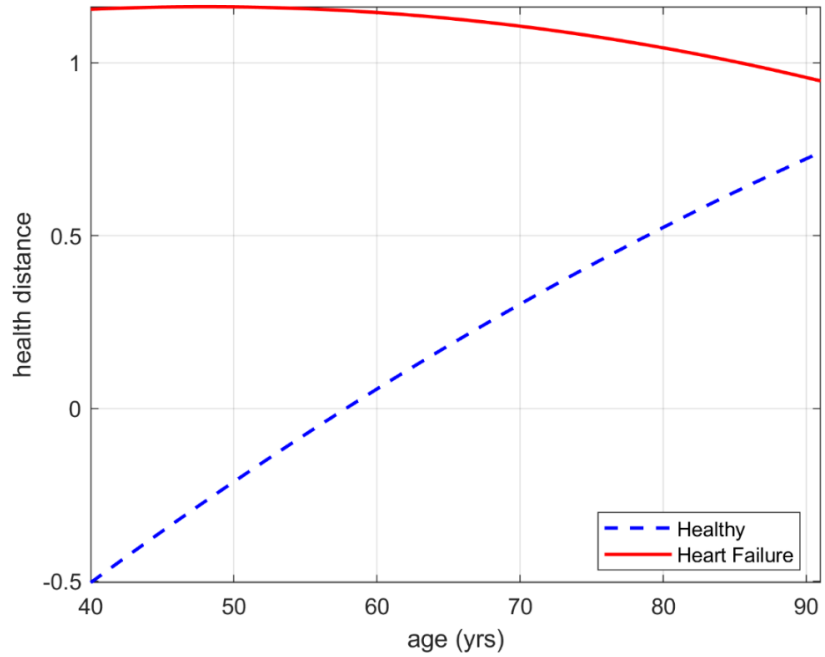
Supplementary Figure S5. Health distance trajectories for Physical activity for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



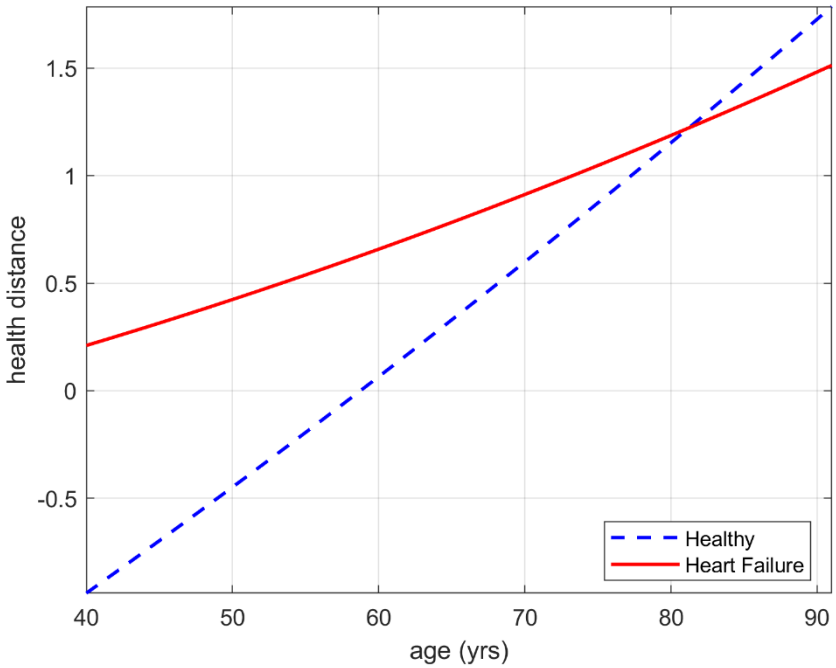
Supplementary Figure S6. Health distance trajectories for General health for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



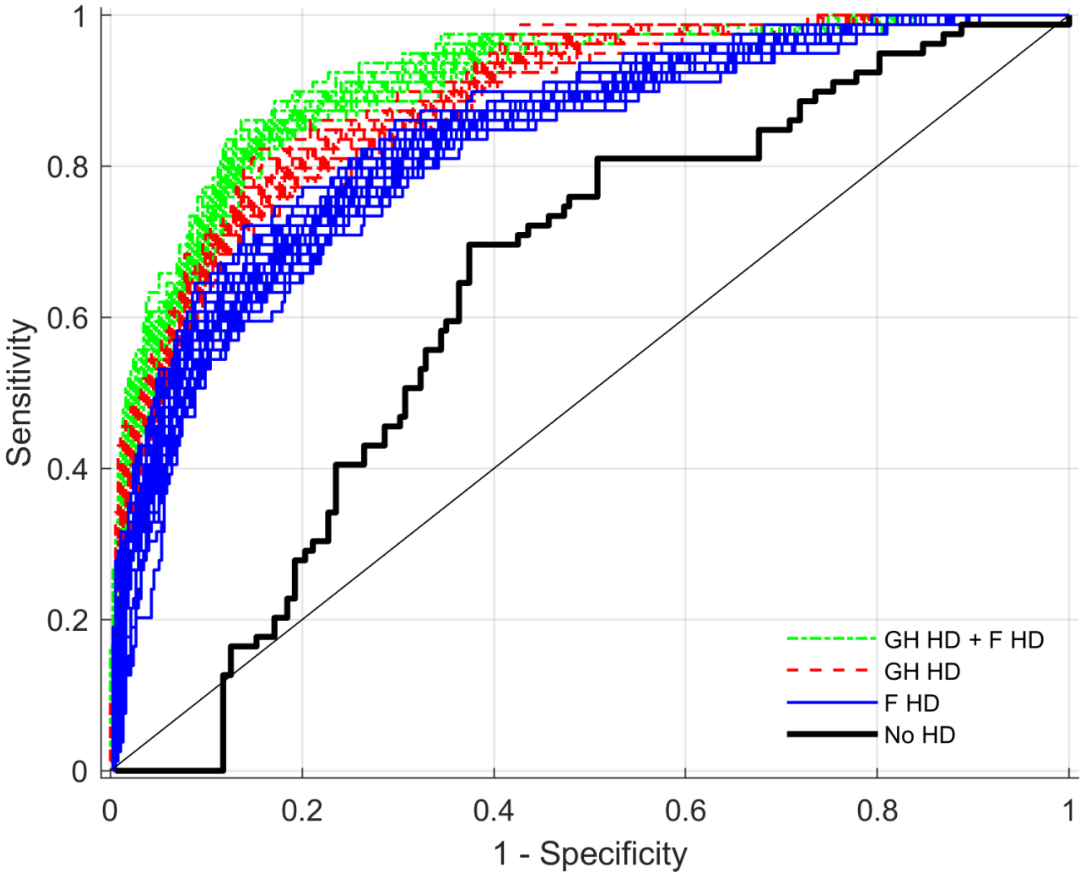
Supplementary Figure S7. Health distance trajectories for Anthropometry for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



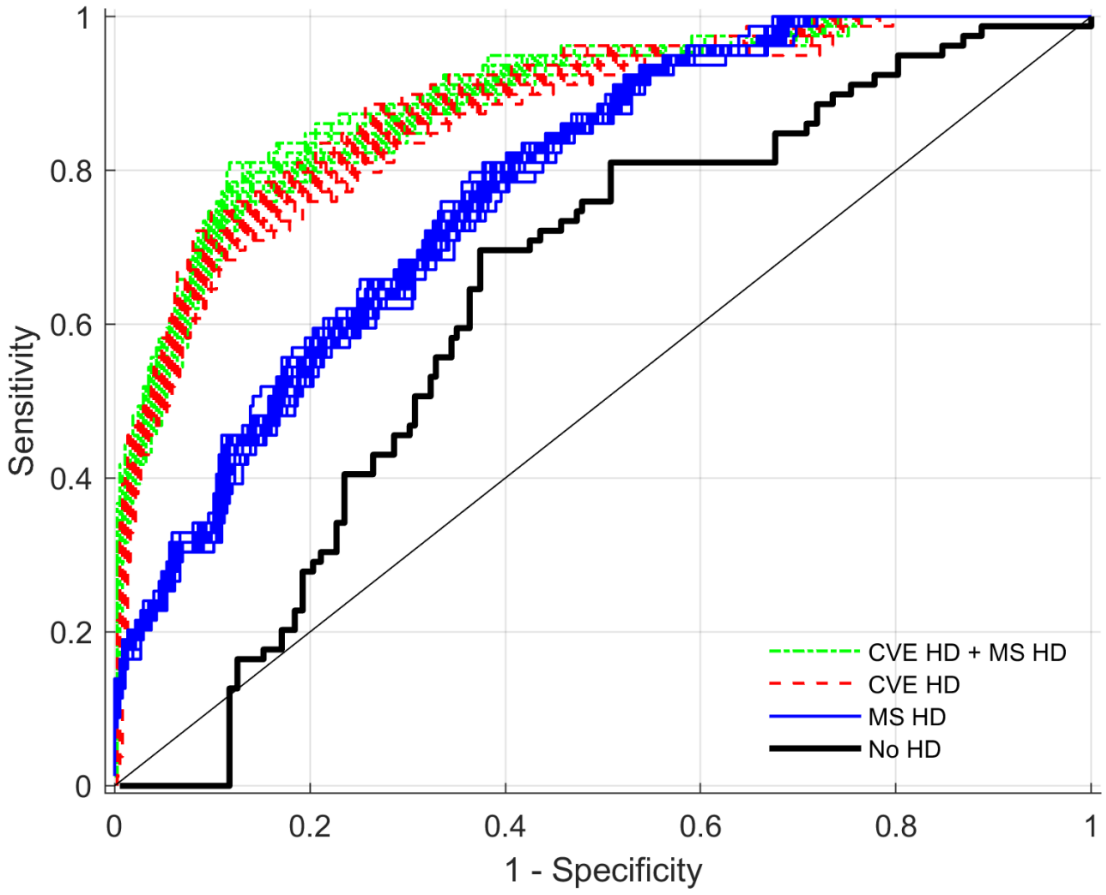
Supplementary Figure S8. Health distance trajectories for Blood biomarkers for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



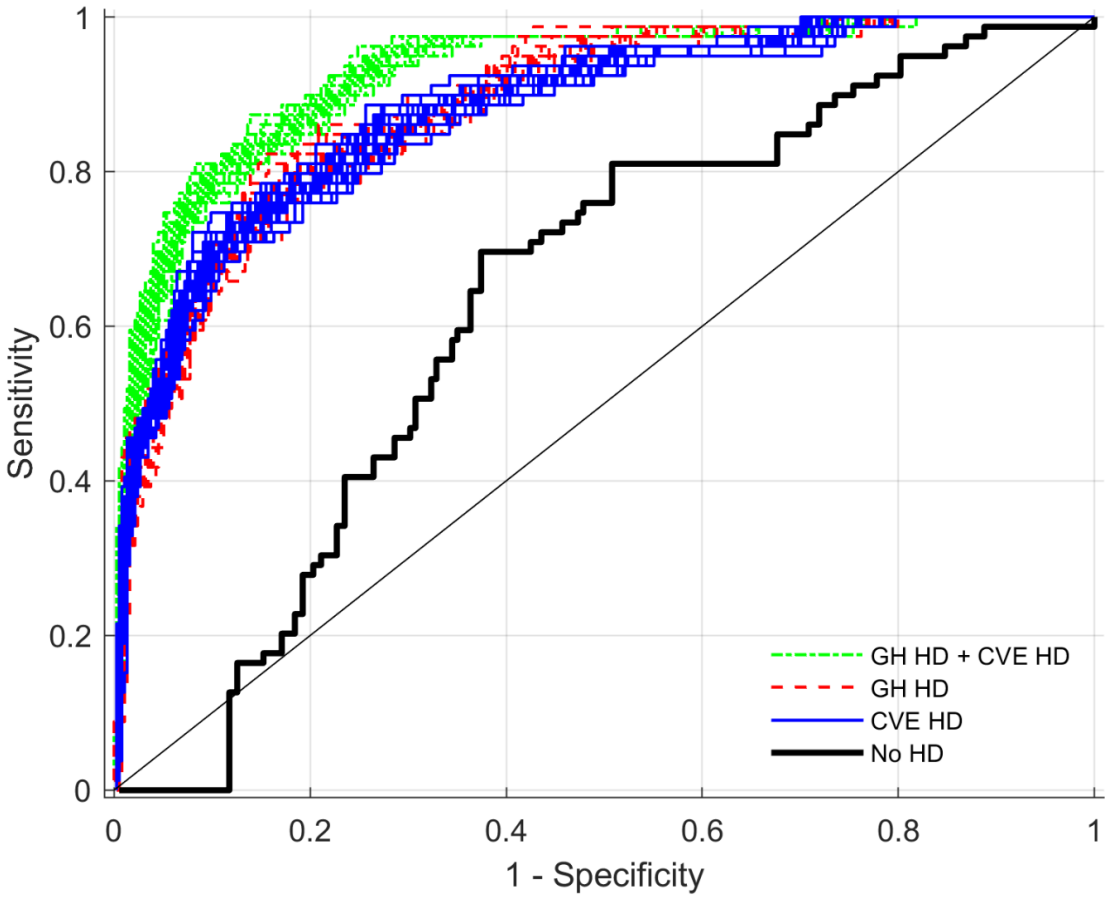
Supplementary Figure S9. Health distance trajectories for Vascular and respiratory health for the Healthy and Heart Failure group presented from 40 to 91 years. The curves correspond to non-smoking females not taking medications.



Supplementary Figure S10. Receiver operating characteristics (ROC) curves for Health distances (HD) of General health (GH), Fitness (F) and the combination of both HDs as predictors of Heart Failure. ROC curves presented for all imputed data sets.



Supplementary Figure S11. Receiver operating characteristics (ROC) curves for Health distances (HD) of Cardiovascular endurance (CVE), Muscle strength (MS) and the combination of both HDs as predictors of Heart Failure. ROC curves presented for all imputed data sets.



Supplementary Figure S12. Receiver operating characteristics (ROC) curves for Health distances (HD) of General health (GH), Cardiovascular endurance (CVE) and the combination of both HDs as predictors of Heart Failure. ROC curves presented for all imputed data sets.

Supplementary Tables

Table S1. *Correlation of biomarkers with age*.

Biomarker	Included in HD	Correlation coefficient	*p*-value
Anthropometry			
Height (cm)	0	-0.291	< 0.0001
Body mass (kg)	1	-0.014	0.7280
BMI (kg/m2)	0	0.197	< 0.0001
WHR	1	0.430	< 0.0001
Body fat (%)	1	0.449	< 0.0001
Lean body mass (kg)	0	-0.265	< 0.0001
Skeletal muscle mass (kg)	1	-0.303	< 0.0001
Vascular and respiratory health			
Rest systolic BP (mmHg)	0	0.412	< 0.0001
Rest diastolic BP (mmHg)	0	0.339	< 0.0001
HR at rest (bpm)	1	0.072	0.0786
baPWV (m/s)	1	0.760	< 0.0001
CAVI	1	0.856	< 0.0001
Preejection period (ms)	1	0.159	< 0.0001
Ejection time LV (ms)	1	0.121	0.0028
FVC	1	-0.582	< 0.0001
FEV1	0	-0.647	< 0.0001
Blood testing			
NTproBNP (pg/ml)	0	0.257	< 0.0001
HbA1c (mg/dL)	1	0.459	< 0.0001
Total cholesterol (mg/dL)	1	0.298	< 0.0001
Triglyceride (mg/dL)	1	0.059	0.1532
HDL cholesterol (mg/dL)	1	0.104	0.0108
LDL cholesterol (mg/dL)	0	0.275	< 0.0001
C-reactive protein (mg/L)	1	0.100	0.0144
Creatinine (mg/dl)	1	0.154	0.0002
Cardiovascular endurance			
Peak $\dot{V}O_2$ (L/min)	1	-0.611	< 0.0001
Peak $\dot{V}O_2$ (mL/kg/min)	1	-0.671	< 0.0001
Peak $\dot{V}O_2$ (mL/kg leanmass/min)	0	-0.668	< 0.0001
Peak O_2pulse (mL/beat)	1	-0.391	< 0.0001
Peak workload (W)	0	-0.628	< 0.0001
$\dot{V}O_2$ at VT1 (mL/kg/min)	1	-0.5369	< 0.0001
$\dot{V}O_2$ at VT1 (L/min)	1	-0.502	< 0.0001
$PETCO_2$ at rest (mmHg)	1	-0.388	< 0.0001
$PETCO_2$ at VT1 (mmHg)	1	-0.581	< 0.0001
$\dot{V}E/\ \dot{V}CO_2$ slope	1	0.295	< 0.0001
$\dot{V}E/\ \dot{V}CO_2$ slope below VT2	1	0.509	< 0.0001
OUES (mL/min)	1	-0.525	< 0.0001
OUES (mL/min/kg)	1	-0.566	< 0.0001

% rel $\dot{V}O_2$ reduction 60sec post test	1	-0.426	< 0.0001
slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	1	0.579	< 0.0001
Peak Lac (mmol/L)	1	-0.689	< 0.0001
Peak $\dot{V}E$ (l/min)	0	-0.551	< 0.0001
Peak HR (bpm)	1	-0.715	< 0.0001
HRR 1 min (bpm)	1	0.159	0.0001
HRR 2 min (bpm)	1	0.403	< 0.0001
Peak exercise systolic BP (mmHg)	1	0.064	0.1433
Muscle strength / power			
CMJ peak power (kN)	1	-0.600	< 0.0001
CMJ height (m)	1	-0.737	< 0.0001
Hand grip strength (N)	1	-0.383	< 0.0001
Hand grip RFD (N/150ms)	1	-0.453	< 0.0001
Isometric leg strength (kg)	1	-0.433	< 0.0001
Neuromuscular coordination			
COP path length (cm)	1	0.652	< 0.0001
Gait speed (m/s)	1	-0.234	< 0.0001
Gait cadence (steps/minute)	1	0.136	0.0010
Stride Length (m)	1	-0.395	< 0.0001
Gait double support (%)	1	-0.053	0.2089
Gait asymmetry (%)	1	0.147	0.0004
Physical activity			
Light physical activity (min/day)	1	0.117	0.0050
Moderate physical activity (min/day)	1	-0.352	< 0.0001
Vigorous physical activity (min/day)	1	-0.277	< 0.0001

Table S2: *Group differences in biomarkers for Healthy (healthy participants aged ≥ 40 years) and Heart Failure (patients with heart failure).*

Biomarker	Included in HD	Healthy (mean)	Reference Population (mean)	*p*-value
Anthropometry				
Height (cm)	0	170.4	174.5	< 0.0001
Body mass (kg)	1	69.8	70.2	0.6885
BMI (kg/m2)	0	23.9	23.0	0.0002
WHR	1	0.9	0.8	< 0.0001
Body fat (%)	1	25	19	< 0.0001
Lean body mass (kg)	0	52.3	56.7	< 0.0001
Skeletal muscle mass (kg)	1	170.4	174.5	< 0.0001
Vascular and respiratory health				
Rest systolic BP (mmHg)	0	28.8	31.9	< 0.0001
Rest diastolic BP (mmHg)	0	130	121	< 0.0001
HR at rest (bpm)	1	79	72	0.8524
baPWV (m/s)	1	61	61	< 0.0001
CAVI	1	13.3	10.2	< 0.0001
Preejection period (ms)	1	8.8	6.2	< 0.0001
Ejection time LV (ms)	1	105.8	98.9	0.0017
FVC	1	313.1	307.6	< 0.0001
FEV1	0	3.9	5.0	< 0.0001
Blood testing				
NTproBNP (pg/ml)	0	138.0	79.2	< 0.0001
HbA1c (mg/dL)	1	5.3	5.0	< 0.0001
Total cholesterol (mg/dL)	1	232	191	< 0.0001
Triglyceride (mg/dL)	1	119	113	0.2969
HDL cholesterol (mg/dL)	1	67.1	62.0	0.0006
LDL cholesterol (mg/dL)	0	130.6	102.6	< 0.0001
C-reactive protein (mg/L)	1	1.93	1.60	0.3875
Creatinine (mg/dl)	1	0.84	0.83	0.2574
Cardiovascular endurance				
Peak $\dot{V}O_2$ (L/min)	1	2.22	3.00	< 0.0001
Peak $\dot{V}O_2$ (mL/kg/min)	1	31.7	42.9	< 0.0001
Peak $\dot{V}O_2$ (mL/kg leanmass/min)	0	41.8	52.9	< 0.0001
Peak O_2pulse (mL/beat)	1	14.1	16.6	< 0.0001
Peak workload (W)	0	182	257	< 0.0001
$\dot{V}O_2$ at VT1 (mL/kg/min)	1	19.5	24.8	< 0.0001
$\dot{V}O_2$ at VT1 (L/min)	1	1.4	1.7	< 0.0001
$PETCO_2$ at rest (mmHg)	1	31.0	32.6	< 0.0001
$PETCO_2$ at VT1 (mmHg)	1	39.7	43.7	< 0.0001
$\dot{V}E/\ \dot{V}CO_2$ slope	1	37.3	34.5	< 0.0001
$\dot{V}E/\ \dot{V}CO_2$ slope below VT2	1	30.5	26.8	< 0.0001
OUES (mL/min)	1	2376	3031	< 0.0001
OUES (mL/min/kg)	1	33.9	43.3	< 0.0001

% rel $\dot{V}O_2$ reduction 60sec post test	1	28.3	33.9	< 0.0001
slope linear $\dot{V}O_2$ off-kinetics (ml/min/s)	1	-13.4	-19.9	< 0.0001
Peak Lac (mmol/L)	1	7.0	10.3	< 0.0001
Peak $\dot{V}E$ (l/min)	0	98	128	< 0.0001
Peak HR (bpm)	1	162	188	< 0.0001
HRR 1 min (bpm)	1	-24	-25	0.8200
HRR 2 min (bpm)	1	-52	-59	< 0.0001
Peak exercise systolic BP (mmHg)	1	190.7	181.3	< 0.0001
Muscle strength / power				
CMJ peak power (kN)	1	2.1	2.9	< 0.0001
CMJ height (m)	1	0.16	0.26	< 0.0001
Hand grip strength (N)	1	344.0	412.7	< 0.0001
Hand grip RFD (N/150ms)	1	226.8	280.9	< 0.0001
Isometric leg strength (kg)	1	104	135	< 0.0001
Neuromuscular coordination				
COP path length (cm)	1	42.0	23.9	< 0.0001
Gait speed (m/s)	1	1.4	1.4	0.1855
Gait cadence (steps/minute)	1	115	113	0.0034
Stride Length (m)	1	1.46	1.50	< 0.0001
Gait double support (%)	1	21.5	21.9	0.1846
Gait asymmetry (%)	1	2.6	2.2	0.0476
Physical activity				
Light physical activity (min/day)	1	103	94	0.0005
Moderate physical activity (min/day)	1	157	178	< 0.0001
Vigorous physical activity (min/day)	1	6	9	0.0041

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Chapter 9

Discussion

Chapter 9 Discussion

This chapter summarizes the main findings of the four publications (1-4) and the two manuscripts that have been submitted for publication (5, 6), discusses them in their scientific context, and provides prospects for future research.

9.1 Synopsis

Figure 1 illustrates the six steps and the six publications of this Ph.D. project. Before designing and conducting the COmPLETE Project, an extensive preparatory phase was performed including writing a literature review on the role of CPET parameters in risk stratification of patients with HFpEF (Step 1 and Publication 1). After identifying the research gaps and a clear need for a research study focusing on the evaluation of physical fitness markers and especially CPET parameters in populations with and without chronic disease, the COmPLETE Project with its two sub-studies COmPLETE-Health and COmPLETE-Heart (Step 2 and Publication 2) was developed. The first of the four manuscripts including original data from the COmPLETE Project covered the evaluation of maximal exhaustion during CPETs, which is a critical and highly discussed topic in the field of cardiopulmonary exercise testing (Step 3 and Publication 3). Step 4 and Publication 4 were the response to the call of the FRIEND registry, a large initiative supported by the AHA, to develop CPET reference values on a global scale. Specifically, Publication 4 provides reference values for the primary outcome of the project, VO_{2peak}, and further maximal and submaximal CPET parameters (including those elaborated in Publication 1), and the association of VO_{2peak} with physical activity. It addresses several shortcomings of previous studies and may pave the way for similar but large-scale and global studies. With the analysis of VO_2-kinetics in healthy individuals and patients with HF (Step 5), the aim was to evaluate further potentially useful CPET parameters for risk stratification. Finally, Step 6 concludes this thesis and involved applying the Mahalanobis distance as a composite measure of physical fitness biomarkers to investigate differences in physiological dysregulation between healthy individuals and patients with HF.

Figure 1: *Illustration of the 6-step process that forms this Ph.D. project.*

9.1.1 Aim 1: To perform a narrative review testing the hypothesis that ventilatory gas exchange variables of CPET are useful for risk stratification and management of HFrEF (1).

The first aim of this thesis was to review the current evidence of CPET variables for risk stratification in HFrEF. The literature review focused on five widely-studied CPET variables and evaluated them based on nine previously-used systematic criteria for biomarkers. For other variables of CPET beyond the chosen five, evidence was not available to meet even a minority of the nine criteria and therefore these variables were not discussed. The included five variables were VO_{2peak} or % predicted VO_{2peak}, VE/VCO_2 slope, EOV, OUES, and $P_{ET}CO_2$. All of these five CPET variables had a thorough proof-of-concept and had been prospectively validated (Criteria 1 and 2). Incremental value (Criterion 3) had been demonstrated for most of the five variables. Further, clinical utility (Criterion 4) had been shown for most variables except EOV and $P_{ET}CO_2$. Clinical outcome (Criterion 5) was demonstrated for all variables except $P_{ET}CO_2$. In contrast, there was a lack of data about the cost-effectiveness of CPET (Criterion 6). Although CPET is easy to conduct (Criterion 7), there was a lack of methodological consensus concerning the preparation, execution, and interpretation of CPET (Criterion 8). Methodological consensus concerning the exercise protocol, exertion criteria, and uniform definitions of the different variables would make studies and datasets more easily and directly comparable and would promote the use of CPET. Some form of reference norms have been published for all five CPET variables (Criterion 9).

A narrow focus on VO_{2peak} persists in the clinical application of CPET. However, as demonstrated in the results above, there is considerable evidence for the value of other CPET variables. The fact that all five variables demonstrated evidence for Criteria 1-3 including “incremental value”, a

multivariable approach integrating the clinical assessment of several and possibly all five variables is recommended. Considering each variable reflects in part a different pathophysiologic feature of HFrEF, combining these variables delivers a broader multi-dimensional picture of the pathophysiologic process and severity of HFrEF. The combined assessment of VO_{2peak} or % predicted VO_{2peak}, the VE/VCO_2 slope, and EOV is recommended as part of the examination. Further, as no additional costs are incurred in investigating the OUES and $P_{ET}CO_2$, they should also be considered as part of routine clinical analysis.

9.1.2 Aim 2: To design a study that can identify physical fitness and cardiovascular biomarkers that best resemble underlying cardiovascular risk with age and to examine which physical fitness markers are impaired with progressing age and in heart failure (2).

The second aim of this thesis was to plan and conduct a cross-sectional investigation consisting of two parts, COmPLETE-Health and COmPLETE-Heart. COmPLETE-Health examined the aging trajectories of physical fitness components and CV properties in a healthy population aged between 20 and 90 years (n ≈ 490). Separately, COmPLETE-Heart assessed the same markers in patients at different stages of chronic HF (n ≈ 80). The primary outcome to determine the difference between COmPLETE-Health and COmPLETE-Heart was cardiorespiratory fitness as measured by CPET on a bicycle ergometer. Secondary outcomes included walking speed, balance, isometric strength, peak power, and handgrip strength. Physical activity as a behavioral component was assessed objectively via accelerometry. Further, CV assessments included pulse wave velocity; retinal arteriole and venule diameters; brachial and retinal arterial endothelial function; carotid intima-media thickness; and systolic and diastolic function. The research performed based on the COmPLETE Project aimed to identify physical fitness and CV biomarkers that best reflect underlying CV risk with age. Further, this research provides data to examine which physical fitness markers are impaired most in HF. The integrative approach allows the definition of new recommendations for diagnostic guidance in aging. Ultimately, this study is expected to offer a better understanding of which functional characteristics should be specifically targeted in primary and secondary prevention to achieve an optimal health span. Only a small subset of the collected data has been analyzed and described in this thesis and Publications 3–6. Further analysis will be performed and will include manuscripts focusing on the vascular parameters, their differences between age groups within the COmPLETE-Health sample, and differences between healthy individuals and patients with early stages of HF. Data presented in this thesis focused on the analysis of the physical fitness parameters of the COmPLETE Project.

9.1.3 Aim 3: To test the hypothesis that high and age-dependent secondary exhaustion criteria are required to balance type I and type II errors in the determination of VO_{2peak} (3).

The third aim of this thesis was formed of three goals: 1) to determine age-dependent cutoff values using tolerance intervals based only on those tests where VO_2 plateaued, 2) to establish a

multiparameter score to improve the performance of a single criterion, and 3) to provide a descriptive analysis of the percentage of participants reaching commonly used exhaustion criteria during a CPET. The percentage of individuals reaching a VO_2 plateau in the current study was 32% and therefore comparable to findings in previous studies. This comparatively large study provided, for the first time, data-based optimal secondary exhaustion criteria for different age groups to allow optimization of the evaluation of VO_{2max}. The suggested criteria were RER 1.13, 1.10, and 1.06; 96%, 99%, and 99% $APMHR_{210}$; and 93%, 92%, and 89% $APMHR_{208}$ for individuals 20–39, 40–59, and 60–69 years of age, respectively. These numbers differed from previously-used cutoffs and our results showed that more stringent criteria need to be applied. Exhaustion criteria recommended by others, with values lower than those recommended here, can underestimate VO_{2max} by as much as 33% on a group level. The proposed multiparameter score with its cut-off of -1.885 provides, for the first time, a meaningful combination of several criteria. The application of this score can, therefore, reduce type I errors compared with using a single criterion with the same small type II error of at most 5%. Previously-used scores selected based on intuition rather than an evidence-based approach result in an unknown number of type I and II errors and therefore are not recommended.

9.1.4 Aim 4: To provide CPET reference values for maximal and submaximal parameters across the adult age spectrum of a healthy European cohort. Further, to test the hypotheses that (1) there is a positive correlation between health- and performance-related CPET parameters and moderate and vigorous PA, and (2) reference values for VO_{2peak} from this healthy European cohort are higher compared to other previously published reference studies (4).

Completing the fourth aim of this thesis involved 1) adding prospectively assessed novel reference values for VO_{2peak} and additional CPET markers of a healthy European cohort by using standardized percentile curves; 2) comparing VO_{2peak} values with the major CPET cycle ergometer reference datasets available; and 3) assessing the associations of objectively measured light PA, moderate PA, and vigorous PA with submaximal and maximal CPET parameters. The study included a healthy sample consisting of 502 subjects aged 20–90 years and represented the first reference dataset (7) meeting 12 out of 14 CPET standards described in the 2003 American Thoracic Society and American College of Chest Physicians statement on exercise tests (8). In the 20-29 year age group, VO_{2peak} values observed were 46.6 ± 7.9 and 39.3 ± 6.5 mL/kg/min for males and females, respectively. The differences in relative VO_{2peak} (mL/kg/min) between two neighboring age decades were between −2% (−0.8 mL/kg/min) and −20% (−6.1 mL/kg/min) for males and between −1% (−0.4 mL/kg/min) and −18% (−5.6 mL/kg/min) for females. Larger differences were observed between the older age categories and therefore the data describe a nonlinear decline. On average, each age category showed a 10% (90% of the previous value) lower VO_{2peak} (mL/min/kg) relative to the next younger age category. VO_{2peak} values of previous studies were, on average, 7.5 mL/kg/min (20%) lower for males and 6.5 mL/kg/min (21%) lower for females. The COmPLETE-Health Study clearly stands out with high VO_{2peak} values, only the Low Land Fitness Registry shows

similar data (9). The large differences seen between these studies' reference values are remarkable and have potential practical and clinically relevant consequences when applying one or the other reference dataset. Further, this study presents the first population-based study to analyze the relationship between objectively measured PA and directly measured cardiorespiratory fitness (VO_{2peak}) in healthy men and women over such a wide age range. We observed strong evidence supporting a positive association between the VO_{2peak} (mL/kg/min) and the level of habitual PA performed at moderate (estimate 0.020; p = 0.003) and vigorous PA (estimate 0.260; p < 0.001). A clinically relevant difference in VO_{2peak} (an increase of ≥ 1 mL/kg/min) (10) was observed with as low as four minutes of additional VPA per day. Further, a 1-MET higher CRF (3.5 mL/kg/min), which corresponds to an approximately 15% lower incidence of myocardial infarction (11), was associated with 13 minutes of additional VPA per day. Submaximal performance-related CPET parameters (VO_2 at VT1 and OUES) showed similar associations with MPA and VPA as VO_{2peak}. These results suggest that across the whole cohort, LPA was too low a stimulus to increase oxygen uptake at the submaximal level (VT1) or enhance oxygen uptake efficiency. As was expected, clinical CPET variables such as $P_{ET}CO_2$ and VE/VCO_2 slope did not show evidence for association with PA levels, which may be explained by the healthy physiological levels of these parameters even in subjects of advanced age.

9.1.5 Aim 5: To test the hypotheses that (1) heart failure patients demonstrate slower VO_2 on- and VO_2 off-kinetics compared to healthy participants, (2) differences between calculation approaches of VO_2-kinetics are prevalent, and (3) VO_2-kinetics can provide additional value beyond that of VO_{2peak} (5).

The fifth aim of the thesis was 1) to examine whether VO_2-kinetics parameters obtained from a CPET can distinguish between healthy participants and cardiac patients with HF and between NYHA functional classes; 2) to determine which VO_2 kinetic parameter and which calculation is most valid; and 3) whether the most promising VO_2 on- and off-kinetic parameter can add additional value to VO_{2peak}. The analysis provided evidence that VO_2 kinetic parameters differ between healthy participants and a group of mild to moderate functionally-impaired patients with HF for all kinetic calculation methods, except for τ VO_2 on-kinetics by VO_2 deficit and mean response time of the ramp-kinetics. The largest z-score difference between healthy individuals and patients with HF was observed for % relative VO_2 reduction 60 s post-test (%) and the slope of linear VO_2 off-kinetics (mL/min/s) of 0.89 (95% CI; 0.59, 1.18) and -0.88 (-1.15, -0.62), respectively. Further, a significant association between VO_{2peak} and off-kinetics was observed (p < 0.001). The slope of linear VO_2 off-kinetics explained 39% of the variation in VO_{2peak} between healthy participants and patients with HF. In contrast, VO_2 on-kinetics showed a significant association with VO_{2peak} but only a modest effect size: the on-kinetics parameter τ VO_2 (s) explained only 4% of the variation in VO_{2peak}. Further, VO_2 off-kinetics can add substantial information to a model predicting health status and disease severity of patients with HF. Thus, VO_2 off-kinetics could be a tool to discriminate not only between healthy participants and those with mild functional impairment (NYHA class I) but also between NYHA classes. Since VO_2 off-kinetics is not

affected by the level of exhaustion, these parameters can be used as a substitute for of VO_{2peak} when maximal exhaustion is not reached or when VO_{2peak} cannot be interpreted. However, additional value beyond that of VO_{2peak} to predict disease severity cannot be provided by VO_2-kinetics. The adequacy index comparing the base models containing age, sex, and VO_{2peak} to a model additionally containing the kinetic parameters was between 0.98 and 0.99. The scale for this index spans from 0 to 1 and an adequacy index near 1 indicates that the base model already provided nearly all predictive information and that the kinetic parameters add little predictive information.

9.1.6 Aim 6: To test the hypothesis that a significant health distance of physical fitness components (i.e., cardiovascular endurance, muscular strength, and neuromuscular coordination) can be observed between patients with heart failure and healthy individuals (6).

The sixth aim of this thesis was 1) to analyze whether differences in composite measures of physiological function (health distance, HD), specifically physical fitness, between healthy individuals and patients with HF can be observed, 2) how HDs change over lifespan, and 3) to determine whether HD *Fitness* can increase discriminative performance between healthy individuals and patients with HF compared to standard clinical biomarkers. The study applied a novel approach from the field of epidemiology and research on aging to physiological biomarkers of physical function. The analysis provided evidence that nine out of ten calculated HDs differed between Healthy and HF ($p \leq 0.002$). The largest HD difference between those groups was observed at the youngest age (40 years); after that, the trajectories of HD *Fitness* of the healthy and HF group continuously approached each other with increasing age. The approaching pattern of HD was observed in most of the calculated HDs by showing negative estimates of the interaction term age by group ($p < 0.05$ for eight out of ten HDs). In both healthy individuals and patients with HF, HD tends towards a common unknown upper limit indicating frailty, disability, or mortality. The predictive performance of HF cases of the base model significantly increased by adding HD *General health* or HD *Fitness* with an increase in the AUC estimate from 0.63 to 0.89 and 0.84, respectively. Compared to the base model, both HD *Cardiovascular endurance* and HD *Muscle strength* biomarkers increase the AUC estimates substantially from 0.64 to 0.88 and 0.78, respectively. There is, however, little evidence that adding HD *Muscle strength* to HD *Cardiovascular endurance* adds value. HD *Cardiovascular endurance* alone reached an AUC of 0.88 compared to HD *General health* with 0.89. Further, there is evidence that the combination of both HD *Cardiovascular endurance* and HD *General health* shows superior predictive power compared to one of the HDs alone.

9.2 General Discussion

9.2.1 CPET Gas Exchange Markers as Clinical Vital Signs

In recent years, several advancements have been made in the field of clinical CPET. The already widely known primary outcome of CPET, CRF measured by VO_{2peak} assessment, has been further strengthened and promoted (11-13). On the other hand, further evidence for additional gas exchange markers has been established and advanced (14). CPET, therefore, has developed into an important tool beyond the evaluation of the single parameter VO_{2peak}. CPET has the potential to provide a multi-dimensional picture of an individual's physiological response to exercise and can unveil underlying mechanisms behind exercise intolerance for several disease conditions. This section discusses the recent advances in CPET as a clinical test, including publications on this topic from the present thesis (Publications 1, 3, 4, and 5).

The Role and Measurement of VO_{2peak} in the Clinical Setting:

VO_{2peak} can deliver important information about an individual's health trajectory and can predict future risk for adverse events and mortality regardless of an individual's current health status, from apparently healthy to a confirmed diagnosis of HF or other chronic diseases (15). Mounting evidence over the last decades has established that low levels of VO_{2peak} are associated with a high risk of CVD and all-cause mortality (11, 12). VO_{2peak} has been shown repeatedly to be one of the leading risk factors for CVD. Some research studies have even described VO_{2peak} as a stronger predictor than established risk factors such as smoking, hypertension, high cholesterol, or type 2 diabetes mellitus (16). In short, individuals showing a high VO_{2peak} are at a significantly lower risk for the development of other risk factors, chronic diseases, adverse events, and even premature mortality (12, 15). Studies also showed an association between health care costs and $VO2_{peak}$ levels (17, 18). VO_{2peak}, therefore, provides comprehensive information to health care professionals when assessing an individual's health status and constitutes a starting point to formulate a plan of care (15, 19).

In 2013, the AHA and the American College of Cardiology (ACC) jointly published new guidelines on the assessment of CV risk (20, 21). The suggested risk calculator of these guidelines, however, did not include CRF in any form—whether predicted by formula, estimated by peak work rate, or directly measured by CPET (VO_{2peak}). This omission was despite the well-described improvement that the addition of CRF to traditional markers significantly improves reclassification of risk for adverse outcomes. Myers (22) criticized in a commentary of the guidelines that they provide a too-narrow focus on the treatment of blood lipids, and that CRF as a risk factor and PA as an intervention and treatment option does not get appropriate recognition. There appears to be a persistence of a narrow focus on clinical markers that can be treated by medications rather than lifestyle interventions. A similar observation was made by Ross et al. (12) in an AHA scientific statement, describing that when a CV specialist performs an exercise test, they tend to focus on ischemic ST-segment displacement and the potential need for coronary revascularization. The measurement and interpretation of VO_{2peak}, however, would deliver essential prognostic value

that is often omitted (23, 24). VO_{2peak} has been shown repeatedly to be a more powerful predictor of adverse outcomes than ST-segment depression or hemodynamic responses assessed in exercise tests (24-27). Reasons for the focus on conventional clinical markers rather than VO_{2peak} may be the result of a clinical cascade of further diagnostic tests and prescribed therapies, which generate income (15, 22, 26, 28). Since the 2013 ACC/AHA guidelines (21), numerous initiatives have promoted the legitimacy of VO_{2peak} assessment as a risk factor. Several statements and studies support VO_{2peak} as a risk factor of similar importance to hypertension or hyperlipidemia and suggest that, as for those risk markers, measurement and treatment of VO_{2peak} should be regularly and widely conducted (15, 22-24). In 2016, the AHA finally provided some recognition of the importance of assessing CRF or VO_{2peak} in clinical practice and described CRF as a "clinical vital sign" (26). However, wide application of VO_{2peak} as a clinical vital sign and PA as a prescription may still require time and a considerable reevaluation by clinicians. In the meantime, the CPET measurement could overcome some methodological burdens in assessing VO_{2peak} with precision in clinical routine.

One of the main challenges of every exercise test is that it is highly dependent on the subject's effort and motivation (14). As many individuals undergoing CPET are not familiar with severe exercise intensities, pushing them to their physiological limit and evaluating whether their physiological limit was truly achieved remains a challenge. It is debated whether the maximal achieved oxygen uptake (VO_{2peak}) can be directly interpreted as a meaningful variable or whether certain exhaustion criteria need to be applied (29, 30). VO_{2peak} has been shown to have high clinical importance even when different exercise modes and protocols were used across a range of studies and whether strict exhaustion criteria were applied or not. Nevertheless, methodological consensus on the evaluation of VO_{2peak} and the availability of easily-applicable tools to evaluate an individual's exhaustion level to minimize the differences between measured VO_{2peak} and mode-specific VO_{2max} could help to strengthen the utility of VO_{2peak} as a clinical vital sign. Evaluating whether a VO_2 plateau was achieved or not remains the only option to distinguish between VO_{2max} and VO_{2peak}. As described in the introduction, however, a VO_2 plateau is observed in only approximately 50% of the tested subjects. In our study, we observed a VO_2 plateau in only 32% of individuals (3). The two applied VO_2 plateau definitions in our study showed a good level of agreement. The more advantageous of the two calculation approaches defined a VO_2 plateau as an increase in VO_2 during the final two minutes that was < 50% of the corresponding increase in the submaximal intensity domain (a full description can be found in the methods section of Publication 3) (3, 31). This approach is recommended when assessing VO_2 plateau status in a population of different age groups and fitness levels. Furthermore, since this approach accounts for the individual increase in VO_2 in the submaximal intensity domain, it can be applied to ramp protocols with different work rate increments. This definition presents a valid, although elaborate, option for an evaluation of whether VO_{2max} was achieved. In the remaining 50 to 70% of individuals undergoing CPET, who do not show a VO_2 plateau, secondary exhaustion criteria present an option to evaluate whether a (near) maximal exhaustion or only a submaximal effort was achieved. If no VO_2 plateau can be observed, the maximally achieved VO_2 should be named not VO_{2max} but VO_{2peak}

(32). If no VO_2 plateau and no pre-specified secondary criteria are reached, the VO_2 measure is submaximal and should not even be described as VO_{2peak}. Publication 3 (3) contributed the first data-based secondary exhaustion criteria for CPET performed on cycle ergometers for a non-athletic sample between 20 and 90 years of age. The suggested criteria are RER_{max} 1.13, 1.10, and 1.06; 96%, 99%, and 99% $APMHR_{210}$; and 93%, 92%, and 89% $APMHR_{208}$ for the age groups 20–39 years, 40–59 years, and 60–69 years, respectively. These numbers differ from previously used cutoffs and our results show that more-stringent criteria need to be applied than previously used. Interestingly, the cut-offs for the youngest age group (20–39 years) do not differ to a large degree from the cut-offs recommended for an athletic population defined using a similar approach (33). Therefore, age seems to be the strongest factor influencing secondary criteria, ahead of VO_{2peak} levels or sex.

A multi-parameter approach was the logical further step as both RER and APMHR have the potential to evaluate whether maximal exhaustion was achieved or not. Previous suggested and applied scores, however, were chosen based on intuition rather than an evidence-based approach and whether they minimize type I and type II errors is unknown. The issues discussed in the introduction for single criteria are also likely to be relevant for multi-parameter approaches. They are often defined post-analysis to minimize the number of participants excluded from a study (34). Our proposed score provides, for the first time, a meaningful combination of exhaustion criteria; specifically, the two secondary exhaustion criteria $APMHR_{210}$ and RER_{max}, combined with age. The application of the suggested score comes with some additional calculation steps, but in research settings, they are justified to decrease type I errors (declaring individuals to have reached their maximal physiological response when they have not) and advance the precision in evaluating VO_{2peak}. Our findings are particularly relevant when interpreting study results that have repeated measurements of VO_{2peak}. Our data demonstrate that, if a criterion is set too low, it would be possible to derive a substantial but fallacious increase in measured VO_{2peak} of up to 32%, on group average, without there being a real change in an individual's cardiovascular and muscle capacity to utilize oxygen, leading to a higher VO_{2max}. Exhaustion criteria are not only highly relevant in studies with repeated measurements but they are also of high importance to the establishment or publication of reference values for CPET. Most published CPET reference values have applied relatively low secondary exhaustion criteria (9, 35-38), such as RER 1.0 or 85% APMHR. Applying these reference values can plausibly overestimate the aerobic fitness of the participant, client, or patient and lead to a misclassification of their aerobic fitness. In the clinical setting, the single RER or HR criteria can provide an easily applicable and time-efficient tool for the clinician to evaluate the level of exhaustion. The criteria $APMHR_{210}$, $APMHR_{208}$, and RER_{max} guide the tester to facilitate further motivation during the measurement since they can be monitored continuously. If an individual did not reach our proposed criteria, VO_{2peak} should be interpreted with caution, however, it still can serve as an approximate estimate of CRF and therefore provide important clinical information (32). In the evaluation of VO_{2peak} progression over time in the clinical setting, the use of exhaustion criteria has a similar utility to that in repeated measurements in research studies. They are of relevance to strengthen the precision of VO_{2peak} tracking over time (33).

Beyond the evaluation of the level of exhaustion, appropriate and precise reference values for VO_{2peak} are needed to improve the accuracy of the interpretation of VO_{2peak} (15, 39). As described in the introduction, CPET variables are only useful when they can be compared to values that are outside the usual spread of values found in health or within the spread of values typically found in disease (40). Knowing an individual's VO_{2peak} expressed as a percentile of what is normal for their sex and age in a healthy population as accurately as possible provides clinicians a reference and benchmark for activity counseling, risk stratification, and therapy decisions (41). The COmPLETE-Health Study provided an opportunity to improve upon existing reference standards for VO_{2peak}. The study addressed several of the shortcomings of previously available reference data sets described in a review by Takken et al. (7), the American Thoracic Society Guidelines, and the AHA Scientific Statement on CPET (8). Improvements included the prospectively planned study design and the population-based approach compared to the use of CPET data from hospital databanks. The strict inclusion criteria, with the exclusion of many types of manifest exercise-limiting chronic diseases and risk factors such as smoking or obesity, improved the precision of the data. Previous studies had excluded cardiovascular disease but not cancer, diabetes, or other circumstances affecting CPET parameters either directly or indirectly through reduced PA (38, 42, 43). Two further novel aspects were the objectively measured and reported PA and the report of several submaximal parameters, both discussed below. Therefore, the COmPLETE Project is a response to the call by the FRIEND Registry to develop accurate VO_{2peak} reference values on a global scale. The data of the COmPLETE-Health Study represent the first reference dataset meeting 12 out of 14 CPET standards, according to the 2003 American Thoracic Society and American College of Chest Physicians statement on exercise tests (8). As described in Publication 4 (4), the comparison of all major reference data sets for VO_{2peak} performed on the cycle ergometers revealed large differences between these studies' reference values. These differences have potential practical and clinically relevant consequences when applying one or the other reference dataset in clinical practice. The observations made in Publication 4 (4), therefore, strengthen earlier similar observations by Kaminsky et al.(43). The COmPLETE Project dataset contributes to addressing the need for CPET reference values from different geographical regions fulfilling high-quality standards.

Importantly and as a logical consequence of the findings in Publication 3 (3), we determined VO_2 plateau occurrence in all potential subjects of the CPET reference value study (Publication 4) (4). For the subjects not reaching a VO_2 plateau, RER_{max} criteria from Publication 3 (3) were applied. Subjects not reaching those criteria were excluded from the reference data set. In line with our expectations, only a relatively small number of subjects needed to be excluded from the COmPLETE Project's reference values (4). As VO_{2peak} was our primary outcome, the examiners were instructed that the assessment of VO_{2peak} had the highest priority and that they should push the participants to their individual maximal exhaustion where no signs for an early termination were present. The applied high and appropriate exhaustion criteria have likely contributed to the higher values compared to previous reference studies (36, 38, 42-44). The large differences

between the reference values shows that the COmPLETE Project was a necessary step to provide accurate reference values for European countries primarily performing CPET on cycle ergometers.

To interpret VO_{2peak} and additional CPET parameters with precision, PA levels of the studied reference population should be known (7). PA is the major behavioral component affecting VO_{2peak} and other CPET markers and remains the main method to increase VO_{2peak}. It is important to evaluate behavioral factors associated with VO_{2peak} to implement intervention strategies. Nevertheless, no previous CPET reference study has reported PA data. The provided reference data set of VO_{2peak} and PA together can help further determine the relationship between these two markers. The study was also able to promote the simultaneous assessment of VO_{2peak} and PA in clinical practice. Knowing both PA and VO_{2peak} can be critical when it is unknown whether a low VO_{2peak} value for an individual is due to severe physical inactivity-related deconditioning or to an underlying pathology. We observed that VO_{2peak} among men and women was higher when they performed more moderate- and vigorous-intensity PA. In particular, activities performed at vigorous intensity had a strong linear association with VO_{2peak}. A clinically relevant difference in VO_{2peak} (an increase of ≥ 1 mL/kg/min) (10) was observed with as low as four minutes of additional VPA per day. Further, a 1-MET higher CRF (3.5 mL/kg/min), which corresponds to an approximately 15% lower incidence of myocardial infarction (11), was associated with 13 minutes of additional VPA per day. These two examples demonstrate the potential beneficial impact of higher-intensity exercise (≥7 METs) in improving CRF and provide further evidence supporting the implementation of higher-intensity aerobic exercise in health promotion.

Intensity may be the most important determinant of PA beyond frequency and duration to improve VO_{2peak}. Several randomized controlled trials have shown the high effectiveness of high-intensity exercise training modalities to improve VO_{2peak} in healthy individuals and diseased populations (45-47). Whether training intervention studies with exactly prescribed training intensities can be compared to our cross-sectional study with PA measures based on accelerometry data needs to be questioned. The average VO_{2peak} values within the age groups separated by sex in the COmPLETE-Health sample are between 20 mL/kg/min and 47 mL/kg/min. This is a large range to draw any conclusion regarding the minimal training intensity or amount of PA needed to observe clinically relevant differences in VO_{2peak} levels. It is widely described that (baseline) fitness levels have a large impact on the PA stimulus needed to elicit changes in VO_{2peak} (26, 48). With VO_{2peak} levels of 32 and 38 in the middle age group (50–59 years), the moderate-intensity PA would correspond to 44–77% and 37–64% of their VO_{2peak} and the vigorous-intensity PA beyond 77% and 64% for females and males, respectively. Our results are supported by earlier findings of training intervention studies in individuals with intermediate baseline VO_{2peak} levels (30–50 mL/kg/min), which reported increases in VO_{2peak} after long-term interventions from training intensities as low as 45% (48, 49). More consistent findings were described when a higher training intensity at 60–80% VO_{2peak} was applied (26, 48, 50, 51). Beyond intervention studies, another population-based, cross-sectional study found a similar association between LPA, MPA, and VPA and VO_{2peak} to ours (52).

VO_{2peak} presents an optimal target for health prevention; however, the associations and mechanisms between PA, $VO_{2peak,}$ and health outcomes are not yet fully understood. Possible explanations of better health outcomes in individuals with high CRF include that these individuals tend to have more cardioprotective CV risk profiles, autonomic tone (potentially reducing arrhythmogenic risk), lower risk for thrombotic events, and improved indices of endothelial function (26). Which of these effects are mediated by PA, and to what extent, needs further research. Some of the mechanisms are likely caused by increased PA. Even though LPA was not associated with VO_{2peak} or any of the other CRF-related CPET variables in our study, PA may yield health benefits independent of improved VO_{2peak}. VO_{2peak} is a very strong but not complete surrogate marker of health and may not reflect all positive effects of PA.

The only small to moderate association between PA and CRF on a population level (53, 54) could in part also be due to the method of measuring PA. The accurate measurement of PA in large populations is especially challenging. Self-reports of PA overrate true values by up to 28% in males and 40% in females (55). Further, accelerometry needs to be applied over several days including both weekend and weekdays, with wear time ≥ 10 h per day considered as compliant wear (56-58). Measuring PA using an objective method over a long duration such as 14 days can help increase the precision of PA assessment on a population level. It is important to report PA data jointly with CPET reference values. It is likely that PA levels vary considerably between geographical regions and assessed populations and have an influence on the reported CPET parameters based on the associations observed in this study. Our presented associations indicate for clinicians the amount of additional PA at a specific intensity level that is needed to increase VO_{2peak} in their individual patients. Furthermore, our study indicates that the GENEActive accelerometry used and cut-offs applied (59) were suitable to assess the associations between PA and VO_{2peak} in a biologically plausible dose-response manner as observed in RCT. Our data can help guide the prescription of preventive PA to increase VO_{2peak} and therefore health outcomes.

Heart Failure: Status of CPET

As described in the introduction of this thesis, there is extensive research and evidence on the role of CPET markers for risk stratification, prognosis, and management of HFrEF, which goes beyond the single parameter VO_{2peak}. The gap between the available scientific evidence and an optimal and wide clinical application may be partly explained by the large number of CPET markers investigated in studies and the variety of definitions and methods applied. The large amount of generated data by a CPET and the nine-panel plot can also be a burden for effective clinical application. Several studies have demonstrated the prognostic value of an individual or several CPET parameters, and scientific statements and reviews have summarized these studies (14, 60, 61). A meta-analysis based on 30 studies meeting the inclusion criteria found that VO_{2peak}, VE/VCO_2 slope, and EOV were all highly significant prognostic markers (diagnostic odds ratios ≥ 4.10) (62). OUES demonstrated promising results as a prognostic marker (diagnostic odds ratio = 8.08) but in only a limited number of studies (n = 2) and subjects (n = 582) (62).

Despite the meta-analysis and scientific statements on the topic, there was still a gap in the literature. To prove that these CPET parameters can be used as surrogate outcomes in patients with HFrEF, several criteria need to be fulfilled. Prospective value, as described by the meta-analysis by Cahalin et al. (62), was just one of nine criteria defined by the AHA and ESC (63, 64). Our review of the literature added novel information on which CPET parameters fulfill which criteria and can, therefore, be recommended for clinical routine application. Our review (Publication 1) (1) demonstrated that the evidence supporting the clinical assessment of variables beyond VO_{2peak} for HF patients with reduced ejection fraction is well established. The five variables, VO_{2peak} or predicted VO_{2peak}, VE/VCO_2 slope, EOV, OUES, and $P_{ET}CO_2$ provide evidence for the criteria "proof of concept", "prospective validation", and "incremental value." Based on the results of the review, the combined assessment of VO_{2peak}, VE/VCO_2 slope, and EOV is recommended as part of the clinical examination. As no additional costs are incurred in investigating the OUES and $P_{ET}CO_2$, they should also be considered as part of routine analysis. A multi-variable approach is, therefore, appropriate.

A criterion not being fulfilled by nearly all variables is "methodological consensus". Although CPET is easy to conduct, methodological consensus on the preparation, execution, and interpretation of CPET and its variables is lacking. International projects promoting the use of CPET, such as FRIEND, have the potential to address this issue and can promote uniform methodological approaches for the five described variables. The lack of methodological consensus hinders the comparison between studies and the establishment of comprehensive reference values. VE/VCO_2 slope values, for example, differ considerably whether they were determined using all data or data up to the second ventilatory threshold. Steps should be taken by large organizations to provide uniform guidelines and scientific statements. FRIEND, for example, could specify which method to use and might even make export sheets available to calculate the variables from the raw data. The cost-effectiveness of CPET should be analyzed not only for VO_{2peak} but also when several markers were applied simultaneously. The cost for a CPET remains the same whether one or five parameters are subsequently used in clinical practice.

Even though the table provided in our review (Publication 1) shows the criteria "reference values" marked as green for all five variables, existing reference values of the submaximal parameters have several shortcomings. To date, reference values or cut-offs provided for these parameters are published in individual manuscripts, using different definitions and assessed in different population groups. No previous publication or database provided all essential CPET variables over a large age spectrum. Some exemplary initiatives have been performed in the Netherlands where several CPET parameters have been reported in a pediatric population (65). FRIEND has started to collect additional measures beyond VO_{2peak} internationally, as well as in the U.S., and seems to be the most promising initiative for the future (66). Our study provides a good current solution as it presents all evidence-based parameters in a large supplemental file in Publication 4 (4).

To appropriately interpret and classify the additional submaximal CPET variables beyond VO_{2peak} requires age- and gender-based normal reference standards. Similar to VO_{2peak}, the OUES,

VE/VCO_2 slope, VO_2 at VT1, and $P_{ET}CO_2$ of an individual are only meaningful if they are considered in terms of what is normal for a given individual if they were healthy. These variables do not decline or worsen at the same rate but do all decline or worsen with age and differ between men and women. For example, a given OUES has a very different meaning in an elderly female compared to a middle-aged male. Knowing an individual's CPET variable expressed as a percentage or percentile of what is normal according to gender and age, with as much precision as possible, appropriately classifies a person in the context of their cardiorespiratory health and performance. Our results also provide essential information for risk stratification and evaluation of interventions. The COmPLETE Project and Publication 4 (4) attempted to determine what constitutes a normal reference standard for all the relevant CPET variables described in the review across 70 years of lifespan from 20 to 90 years of age for males and females. The COmPLETE-Health Study was planned following the available guidance for an optimal set of normal values (7, 8). Specifically, the COmPLETE-Health Study is the first study (9, 67) reporting CPET parameters using a cycle ergometer and fulfilling almost all criteria named by these guidelines, and provides several markers beyond VO_{2peak}. Dichotomous cut-offs seem an easy solution for a user-friendly application for some CPET variables such as those proposed for OUES, VE/VCO_2, or $P_{ET}CO_2$. A VE/VCO_2 slope of 34, for example, is often proposed as a cutoff to evaluate the prognosis of HF patients (68). Based on this cutoff, 17% of participants older than 60 years of age in our cohort would have been classified as high-risk patients, despite being free from heart diseases or any other exercise-limiting condition. Similar observations could be made for OUES or $P_{ET}CO_2$ in the age groups 70 years of age and beyond. Overall, the application of dichotomous and age-independent cut-offs of CPET parameters in older and, in particular, very old subjects must be regarded as critical for risk stratification. Therefore, the use of quantile curves as provided by our study should be used or alternative age-corrected dichotomous cutoffs could be discussed.

Similar to the selection of VO_{2peak} reference values, each exercise laboratory must select an appropriate set of reference data that best reflects the characteristics of the individuals tested, exercise mode applied and PA levels considered for submaximal CPET variables. Reporting submaximal and additional CPET variables beyond VO_{2peak} in reference data sets is likely to lead to a wider application of these parameters in clinical practice. Our data set presents adequate reference values for a European population and provides an exemplary comparative basis for answering important questions concerning the normalcy of exercise responses in individuals and patients, and can significantly impact the clinical decision-making process.

For other variables of CPET beyond the five described in our review (Publication 1), such as the O_2 pulse trajectory, VO_2/work trajectory, circulatory power, VO_2 at VT1 and VO_2 onset and recovery kinetics, evidence was not yet available to meet even a minority of the nine discussed criteria to be recommended as surrogate markers in HF patients at this point. Of these variables, VO_2-kinetics seemed, however especially promising for risk stratification and prognosis in patients with HF. According to previous evidence, it might be suitable when maximal exercise is not reached (69, 70). A recent study showed that the determination of the VO_2 off-kinetics is independent of the

level of exhaustion (71). Furthermore, the evaluation of the on-kinetics only needs submaximal effort and can be determined from the resting period to a submaximal load. We, therefore, investigated VO_2-kinetics as a potential parameter for risk stratification and analyzed it in our COmPLETE-Health and COmPLETE-Heart sample. VO_2-kinetics are traditionally measured by performing several constant load tests. Such tests are very time consuming and cost-intensive and provide only one single clinical parameter (VO_2-kinetics). CPET using a ramp protocol is the preferred method to perform an exercise test in the clinical setting (14) and provides a clinician with a large amount of clinically relevant data, such as described in Publication 1 (1) including VO_{2peak}, VE/VCO_2 slope, OUES, EOV, $P_{ET}CO_2$ as well as an electrocardiogram and blood pressure response to a maximal exercise test. Publication 5, therefore, focused on the utility of VO_2 -kinetics during a ramp protocol rather than performing further research using constant load protocols.

It is highly unlikely that an additional exercise test would be performed in clinical routine practice. We wanted to investigate whether VO_2-kinetics could be valuable as an additional marker or as a substitute for VO_{2peak} without requiring additional work and financial cost. Publication 5 (5) showed that VO_2 off-kinetics have a greater difference between healthy participants and patients with HF than VO_2 on-kinetics and ramp kinetics. Furthermore, off-kinetics were strongly associated with VO_{2peak}. The best on- and off-kinetic parameters significantly improved a model to predict disease severity according to NYHA class in patients with HF. If VO_{2peak} cannot be determined, VO_2 off-kinetics provides an acceptable substitute. This is a novel and relevant finding for clinical application in practice. However, additional value beyond that of VO_{2peak} cannot be provided by VO_2-kinetics. Based on our results it can be suggested that VO_2 off-kinetics could be used as an alternative measure of aerobic function when maximal exercise criteria are not reached and VO_{2peak} cannot be interpreted. Previous research showed that the level of exhaustion had no impact on VO_2 off-kinetics (71) and therefore VO_2 off-kinetics can also be reliably determined in tests with submaximal efforts. VO_2-kinetics, however, do not present a 1:1 substitution for VO_{2peak} but provide an important measure of the CV system's ability to rapidly increase or decrease the perfusion of the working muscles (72, 73) and the ability of the muscles to change the rate of oxygen utilization rapidly (74-76). Therefore, critical information about the regulating capacity of the cardiovascular system and the skeletal muscles can be gained from the determination of VO_2-kinetics (72, 73, 75). Furthermore, VO_2-kinetics are relevant to daily life. Most daily PA and mobility tasks performed require submaximal, rather than maximal, oxygen uptake. Individuals of all age groups undergo countless metabolic transitions daily to submaximal VO_2 (77). It is therefore not surprising that VO_2 off-kinetics were able to predict disease severity according to NYHA classes in Publication 5 (5). In mobility- or exercise-impaired older adults, such as HF patients, VO_2-kinetics might be even more relevant to objectively evaluate exercise limitations and functional declines compared to maximal aerobic exercise capacity (VO_{2peak}). Further research, however, is needed to confirm this hypothesis. Our study could not confirm that VO_2 kinetics is superior to predict disease severity over VO_{2peak}. Studies such as that by Alexander et al. (78) that focused on functional mobility, compared to disease severity as in our study, showed that in mobility-impaired older adults without HF, VO_2-kinetics during the onset of and recovery from submaximal

exercise were more predictive of functional mobility than VO_{2peak} (78). Further, the studied population is likely to have an impact on the results. It could be interesting to analyze outcomes of functional mobility in the patients with HF in our COmPLETE-Heart Study, such as comfortable gait speed, or outcomes of health-related quality of life as assessed by SF8.

Based on our results and previous findings, VO_2-kinetics could be a potential critical variable to target in primary or secondary prevention. This could lead to an increase in exercise tolerance, functional mobility, and quality of life in elderly individuals and patients with HF. Evidence regarding the effects of treatment strategies targeting VO_2-kinetics is surprisingly scarce. There is some evidence that following heart transplantation in patients with severe HF, VO_2-kinetics were increased only in a subgroup of those patients having the slowest VO_2-kinetics, despite VO_{2peak} increasing in the whole group (79). Further, there is initial evidence that VO_2 off-kinetics may be improved in HF patients by employing inspiratory muscle training (80) but not following two months of high-intensity residential exercise training (81). Pharmacologic treatment with angiotensin-converting enzyme (ACE) inhibitors and beta-blockers failed to improve VO_2-kinetics to the level of healthy age-matched controls (82).

The lack of methodological consensus is a considerable burden for further development of VO_2-kinetics. Conflicting results from previous studies are likely to be caused by the analysis of varying aspects of VO_2-kinetics and because reproducibility differs between calculation approaches (83, 84). VO_2-kinetics were determined in previous studies from the on-transient behavior following a rapid increase of exercise intensity (i.e. on-kinetics) (70, 85, 86) from the recovery period of a constant load exercise or incremental CPET (i.e. off-kinetics) (69, 86-90), or from the initial delay at the beginning of a ramp test (i.e. ramp test-kinetics) (91). Different calculation approaches were also used, including linear and non-linear least-squares method regression analyses (86, 87, 89, 90), VO_2 deficit and the amplitude of the on-transient (70, 85, 92), and the percentage decrease of VO_2 at a given time in the recovery phase (69, 88). Our results further support the assumption that the assessment method and quantification play a crucial role. In investigating all major approaches to measure VO_2-kinetics by a ramp protocol, we believe that we could partly solve the confusion on which approach to apply. Our results indicate that VO_2 off-kinetics, irrespective of the calculation method, discriminate better between healthy participants and patients with HF compared to VO_2 on-kinetics. The largest differences between healthy individuals and patients with HF were observed in off-kinetics parameters determined from the first minute of the recovery period only (% relative VO_2 reduction 60 s post-test and slope of linear VO_2 off-kinetics). Therefore, further studies should apply one of these two methods, focusing on the very early phase of the off-transition following a standard ramp protocol.

Similar to other CPET markers, we found that VO_2-kinetics change with age, so age-stratified reference values are also of importance for this variable. To date, changes in on- and off-kinetics with increasing age of the participants have only been reported in a small number of studies and have not been quantified over such a large age span and in such a large cohort as COmPLETE-Health (93). On- and off-kinetics tends towards pathological numbers with increasing age. The

deceleration of the VO_2-kinetics with increasing age could potentially be explained by an impaired ability to increase cardiac output during exercise (94) and the resulting reduced muscle blood flow together with a decline in muscle capillary density and capillary-to-fiber ratio as well as mitochondrial density (95, 96). The reduced ability of arterioles to dilate could be responsible for the compromised O_2 delivery, as shown in animal models (97). Also, microvascular alterations could contribute to a reduced ability to increase arterial-venous O_2 extraction. Knowledge of the physiological response and variations in healthy individuals is critical for an optimal interpretation of kinetic parameters. The presented quantile curves in Publication 5 (5) can serve as reference values and could increase the sensitivity and specificity of these kinetic parameters, in line with the other CPET variables, to detect a pathological response to exercise.

9.2.2 Physical Fitness Markers beyond CPET

The primary goal of the COmPLETE Project was to assess physical function across the lifespan in healthy individuals and patients with HF. As described in the introduction, the assessment of physical fitness components in research and clinical practice has either primarily focused on CRF, particularly in the field of cardiology, or on frailty measures such as gait speed or handgrip strength in geriatric medicine. The COmPLETE Project assessed, for the first time, all major physical fitness components—endurance capacity, muscle strength/power, and neuromuscular coordination—in a patient sample with HF and a cohort of healthy individuals across lifespan. Manuscript 6 (6) aimed to combine those fitness markers to composite outcomes termed health distance (HD) by applying the Mahalanobis distance (98, 99). We constructed HDs based on 48 biomarkers and formed domain-specific sets of biomarkers. The most notable findings and their relation to the other manuscripts of this thesis are discussed here.

HD *all biomarkers* and HD *Fitness* provide composite outcomes that decline with age in healthy participants and that discriminate between healthy and patients with HF. The combination of relevant biomarkers can, therefore, increase the precision of overall physical fitness assessments compared to individual biomarkers because HD is less susceptible to measurement errors or deviation of the norm of only a single biomarker. HD *all biomarkers* including all biomarkers provides insight into physiological dysregulation with increasing age in health and HF. Precise and multidimensional information regarding physiological dysregulation and reductions of physical fitness due to early stages of diagnosed or undiagnosed disease can be drawn by such a composite outcome (100, 101). HD *Fitness* could help to timely intervene in age- or disease-related physical fitness reductions by observing deviations of the norm before single parameters could do.

Adding “non-relevant” markers to the composite outcome HD for the given purpose, such as risk stratification, discrimination of healthy aging and early stages of disease occurrence, or prediction of future risk, likely worsens the overall performance of the outcome, as shown in our results and previous findings in the field of genetics (102). The addition of “non-relevant” markers could lead to less powerful composite measures compared to an HD with fewer but only relevant biomarkers or even less powerful ones compared to a single relevant biomarker. The initial steps in the

establishment of a new biomarker remain, therefore, the same and are still essential. The biomarker must fulfill several criteria, such as those defined by Hlatky et al. (63) and Vlachopoulos et al. (64) and applied to the CPET parameters for risk stratification and management of HF in publication 1 (1). Only when an outcome fulfills such criteria should it be included in a composite measure, such as the HD. Adding novel and potentially "relevant" parameters that do not provide a wide evidence base yet as a surrogate outcome could improve but also harm a composite measure's overall performance. Therefore, an evidence-based assessment of the included markers seems essential when deciding whether to include biomarkers in HD. This process and the inserted biomarkers are the foundation of the overall composite outcome HD's performance.

Various technological advances have been made in recent years in the field of physical fitness assessment. These improvements include the breath-by-breath analysis, which became standard when performing CPET (14). Further, novel technologies, such as inertial sensor systems for gait analysis, increased the number of directly available gait parameters following a simple gait test in a standard corridor (103-106). Compared to previous analyses in gait laboratories, these measurements are inexpensive and time efficient (103). Further, compared to gait speed assessment by a hand stopwatch, gait analyses by inertial sensor systems delivers a large amount of data. In addition, more user-friendly strength assessment tools, such as easily operable force plates and digital handgrip strength dynamometers, have been developed. All these technical developments support the more efficient measuring and processing of a large number of physical fitness markers for both research and clinical applications.

However, and as described above, only biomarkers with a substantial evidence base for the given purpose should be used in clinical practice, whether they are applied as single markers or included in a composite outcome, such as HD. It has been repeatedly shown that "relevant markers" combined to composite outcomes are more powerful predictive measures than single biomarkers (100, 107, 108). Further, HD can simplify the quantification and interpretation of physiological dysregulation and physical function decreases. HD *Fitness* or HD *General health* also appear to be attractive outcomes to target in intervention trials because they describe overall physiological function and physical fitness.

Based on the results of publication 6 (6), CPET as a tool for evaluating physical function in a healthy population and patients with HF was further strengthened. HD *Cardiovascular endurance*, which included several parameters all assessed by CPET, was superior to HD *Fitness*, which included all HD *Cardiovascular endurance* parameters but also muscle strength and neuromuscular coordination parameters. Based on these results, it can be argued that CPET parameters remain the preferred test if the aim is to collect physical fitness biomarkers that can discriminate between healthy and patients with HF. These results agree with the literature described in the introduction and discussion sections covering the important role of CPET markers and, in particular, CRF as clinical vital sign (26).

Beyond CPET markers (HD *Cardiovascular endurance*), the muscle strength composite outcome distinguished between healthy individuals and patients with HF. If CPET cannot be performed, the assessment and combination of several strength markers could be a rapid and efficient (but less powerful) alternative in clinical practice for assessing at least some physical fitness aspects. The willingness of the subjects and patients to perform strength assessments is generally higher compared to a CPET. The shorter time of the effort required and the possibility to perform the test in everyday clothes are likely leading to the increased strength assessment acceptance. Neuromuscular coordination is affected in both groups of individuals with and without HF by aging to a similar extent and, therefore, did not discriminate between both groups (6). Measures of gait outcomes and balance, the components of HD *Neuromuscular coordination*, are likely to deliver some information regarding frailty in particularly old and/or multi-morbid patients, as shown in previous studies (109, 110) but not in our study sample.

The HD *General health*, a combination of different classical biomarkers such as various blood markers, body composition, and parameters of lung function and vascular health created a novel composite outcome that increased with age in both healthy and HF patients and differed between both groups in all age decades. One of the most decisive results of the analysis included in publication 6 (6) is that the addition of HD *Cardiovascular endurance* to the base model with age and sex and the HD *General health* was not only significant, but it showed a clear improvement in discriminative performance beyond the full set of comprehensive clinical biomarkers (HD *General health*). These results strongly support the assessment of physical fitness markers and, specifically, the performance of CPET in the clinical assessment of apparently healthy and HF patients.

9.3 Strengths and Limitations

This Ph.D. project has several strengths, but also some limitations that should be considered when interpreting the results and considering future studies. A major strength is that compared to other studies, CPET was our primary investigation, and VO_{2peak} our primary outcome. Therefore, the evaluation of CPET markers was planned with a lot of attention to detail such as choosing an optimal protocol, performing regular quality checks, performing calibrations before every single test, only deploying a small number of well-trained investigators, and applying very strict procedures. The COmPLETE-Health Study is also the first to evaluate VO_{2peak} over such a large age range of 20-90 years. Previously, reference data for individuals 80–90 years of age had been lacking and will become important considering the increasing number of individuals in this age group. The COmPLETE-Health Study made a considerable effort to extend the data up to 100 years of age, but beyond 90 years of age, potential subjects fulfilling the strict in- and exclusion criteria are extremely limited.

The COmPLETE-Health Study was planned prospectively to create reference values for CPET variables and additional physical fitness and CV outcomes. It has, therefore, several advances over other retrospective analyses of available data sets. In particular, retrospective reference values from hospital-based data banks need to be interpreted with caution. Participants in hospitals are prone to have a clinical indication (e.g., shortness of breath) to perform diagnostic and prognostic

tests such as CPET and others. Even though the included participants of other studies have not been diagnosed with CV disease, they are likely to have presented with concerns about physical performance or exercise tolerance. Furthermore, we had a balanced distribution of subjects across all age decades and 50% of the participants were women. This is the first time that a study measuring VO_{2peak} and presenting reference values has objectively measured PA by accelerometry. The evaluation of PA as the primary non-inheritable factor was described in a recent review (7) s an essential shortcoming of earlier studies. PA assessment provides further insights into the highly discussed topic of the relationship between PA and CRF on a population level. The excellent adherence to the accelerometer measurement and the availability of PA data for 95% of the subjects for 10 or more days further strengthens the PA assessment in this study. The applied in- and exclusion criteria were strict. Additional health markers, especially CV markers and blood analysis, were assessed and can be used as additional exclusion criteria in further publications if the analyzed markers seem to be susceptible to certain risk factors. A further strength was the evaluation of physical exhaustion of the individuals undergoing CPETs by elaborative analysis of VO_2 plateau status using several definitions and secondary exhaustion criteria. Previous studies on this topic have been limited to a relatively small number of subjects. The determination of VO_2-kinetics according to all suggested methods in a study sample of more than 600 subjects is a noteworthy strength. Several studies have either measured CRF or have performed some kind of functional mobility battery in elderly individuals, but to date, no large scale cohort study has assessed all major health-relevant physical fitness components: cardiorespiratory fitness, muscular strength, and neuromuscular coordination.

The collaboration with Duke University to use novel statistical tools for composite measures and the application of these for the first time to combine several physical fitness markers is a novel approach in health and exercise science. Further, beyond the wide variety of physical fitness markers assessed, in-depth vascular phenotyping was performed including microvascular and macrovascular analysis. These results have the potential, in analysis beyond this thesis, to understand the associations between early vascular aging and various physical fitness markers in healthy individuals and patients with early stages of HF.

Limitations of the COmPLETE Project include a selection bias through our recruitment strategy. Considering an inclusion rate of 3% to 5% of invitations sent, it is likely that our study participants had improved physical function and health status relative to those individuals who received an invitation but did not take part in the study. However, the study's goal was not to recruit a representative sample of Swiss citizens but rather a sample of healthy male and female individuals who fulfill our inclusion criteria across age groups. We believe, therefore, that the potential selection bias is a negligible problem considering our aims. Further, it should be noted that response and inclusion rates similar to ours have been observed in other recent cohort studies (111).

The provided reference values may only be of the highest accuracy for a relatively small geographic region and lifestyle behavior, as PA or other aspects that could affect health or physical

fitness markers could differ between regions. The report of PA data together with the CPET variables does restrict this limitation to a minimum as the clinician can interpret the CRF values in the context of PA data. A further limitation is that the cross-sectional design does not allow for conclusions to be made about causality for the presented associations in this thesis. The associations observed between PA and CPET parameters could also be interpreted to indicate that the individuals who are capable and motivated to exercise more vigorously tend to be those who naturally have a higher CRF. A follow-up study in three to five years could provide further insights into the association of PA levels and CRF. Another limitation is the use of absolute intensity cut-offs for PA (sedentary, < 1.5 METs; light, 1.5–3.99 METs; moderate, 4.00–6.99 METs; or vigorous, ≥ 7 METs) for all participants irrespective of the wide range of fitness levels in the sample. Defining PA on a relative scale based on VO_{2peak} may be more suitable because older and unfit individuals are very likely to have problems reaching certain MET levels, which may be close to their maximal aerobic capacity (i.e., 7 METs, 24.5 mL/kg/min). Relative PA intensity cutoffs by individual maximal VO_{2peak} or $VO_{2reserve}$ may, therefore, be a more valuable tool for interpretation of the intensity of PA in heterogeneous populations (112). However, the presented association between PA and VO_{2peak} is adjusted for age, which is likely to solve this issue to a large degree.

The changes in physical fitness parameters over age decades need to be interpreted with caution in COmPLETE-Health. A previous study indicated that longitudinal investigations found larger decreases in biomarkers compared to cross-sectional investigations (113). However, we wanted to describe the optimal and healthy aging path of these markers, and this is likely to be approximated by the cross-sectional design.

A further limitation of this study is the seasonal fluctuations, which can influence CPET parameters and PA levels (114). The COmPLETE-Health Study was undertaken continuously over 1.5 years, and a random distribution of participants to the seasons can be assumed. Although as the assessment period spanned over 1.5 years, some of the seasons are overrepresented. Since the seasonal variation affects both the PA and CPET parameters it can be argued that the associations between those variables have not been meaningfully affected. Circadian fluctuations also influence physical fitness markers such as VO_{2peak} (115) and muscular strength outcomes (116). Depending on their circadian preference and chronotype, individuals can achieve their peak performance at different times of the day (117, 118). Since the CPET and the other physical fitness tests were performed between 10:00 and 18:00, not all subjects likely reached their maximum performance level.

The COmPLETE-Health datasets did not provide an optimal study design to evaluate evidence-based secondary exhaustion criteria. In an ideal scenario, it would be known which of the individuals had reached their individual maximum and which did not. As many of the individuals not reaching a VO_2 plateau still reach their maximal physiological limit (but we do not know which ones), the classic two-by-two table and the subsequent calculation of sensitivity and specificity of the different cut-offs could not be calculated. Nevertheless, the criteria recommended in Publication 3 (3) can limit type II errors to a maximum of 5% and can limit type I errors compared to lower or no exhaustion criteria.

For the two manuscripts that include the dataset of COmPLETE-Heart, a limitation is that we were able to recruit patients with only mild to moderate-severe HF. When investigating suitable parameters that can distinguish between healthy aging and HF, such as those in Publication 5, this might not be a significant limitation, because the distinction between mild HF patients and healthy elderly is the most difficult task. To research the effects of HF on various aspects of CV physiology and physical fitness, however, more severe cases would be advantageous. A further limitation is that the participants were not characterized or sub-grouped into HFrEF, HFmEF, and HFpEF according to the European Society of Cardiology guidelines. Echocardiographic data have been assessed but not yet analyzed. Furthermore, the sample size of 79 would likely be too small to perform an analysis using subgroups within the COmPLETE-Heart sample. HFpEF remains a major challenge in health care and further insight into exercise limitations, physiology, and potential markers advancing the management among these patients would be of high relevance.

The investigation of parameters that can distinguish between patients with a chronic disease such as HF and healthy age-matched counterparts is important but equally crucial is to evaluate potential parameters for risk stratification among the physical fitness markers, follow longitudinal data and determine hard endpoints. A follow-up study over several years could provide such data.

The selection of physical fitness measurements was made based on their objectivity and validity and reliability, and also on the costs and practicalities of carrying out the measurements in clinical practice. A limitation of this study was, therefore, that we did not include the gold-standard assessment method for muscular strength using an isokinetic dynamometer. A wide clinical application of this measurement would be highly unlikely due to its high time and cost requirement. An additional application of the motor toolboxes described by the NIH (119) would have been useful to compare not only CPET markers but also muscle strength/power and neuromuscular coordination measures to other data sets.

9.4 Prospects for Future Research

Publication 4 was the answer to the call to develop CPET reference values on a global scale by the FRIEND initiative supported by the AHA. We showed that accurate CPET reference values are important to determine CRF with precision. Additional high-quality CPET datasets similar to that of COmPLETE-Health are now needed across all geographical regions. A planned next step is to merge the COmPLETE Projects CPET database with the FRIEND registry. To achieve the high goals set by FRIEND, the registry needs to develop from a national to an international registry covering CPET data performed on both cycle ergometers and treadmills. Recently, the first global reference standards were published by FRIEND for treadmill testing (66). An open-source database of CPET reference values might be the optimal solution to create precise and updated CPET reference data across the globe (7). Researchers, end-users, and industry should collaborate and establish clear and strict criteria for data sets to be included in the online database. A simplified and automated upload of anonymous CPET data through the CPET metabolic cart's software would simplify and promote such an initiative. This approach would also allow reference values to be updated continuously. This is important as lifestyle-related behaviors of population groups can change over

time and affect CPET values. Further, FRIEND could benefit from extending the stored and published data from VO_{2peak} data to additional submaximal CPET parameters such as those described in Publication 1 (1). In addition, health and behavioral characteristics assessed simultaneously to the CPET assessment could lead to further insights into ways to promote behavioral strategies to increase VO_{2peak}.

The applied approach to generate optimal secondary exhaustion criteria for maximal exercise in Publication 3 (3) should be extended to other population groups. The focus here should be on disease populations undergoing regular CPET in clinical practice such as HF and COPD. It would also be valuable to investigate whether criteria established through bicycle testing are transferable to treadmill testing, or whether alternative criteria are needed. An implementation of an automated VO_2 plateau detection according to the definition used in Publication 3 (3) into the software of CPET metabolic carts would save practitioners and researchers time and effort. The automated VO_2 plateau detection would improve the evaluation of whether an individual's maximal physiological limit was reached. Further, when no VO_2 plateau is present, the recommended multiple criteria approach from Publication 3 (3), using the secondary exhaustion criteria RER_{max} and HR_{max}, could be straightforwardly integrated into a range of software. These technical advancements could help the application of maximal exhaustion criteria in research and clinical settings.

Further studies are needed to investigate VO_2-kinetics (especially VO_2 off-kinetics following a ramp protocol) in longitudinal studies to evaluate the sensitivity of these parameters to track changes with increasing disease severity and age. The potential of VO_2 off-kinetics to predict hard endpoints such as hospitalization or even mortality would be a logical next step after observing that VO_2 off-kinetics can distinguish between healthy individuals and patients with HF, and between disease severities. The practicality of a longitudinal investigation involving a follow-up study of the COmPLETE-Heart sample needs further consideration. Due to the relatively low number of HF participants and the high number of mild to moderate HF cases, it is likely not the optimal sample. Longitudinal changes in healthy individuals, however, could feasibly be determined by a follow up of the COmPLETE-Health sample. The reliability of the two suggested methods to determine VO_2 off-kinetics (% relative VO_2 reduction 60 s post-test, slope of linear VO_2 off-kinetics) should be investigated. Further, the suggested VO_2 off-kinetics parameters could be implemented by the CPET cart software and deliver clinicians additional markers without any additional time or cost spend.

In summary, the potential technological advancements by integrating some of the findings of this thesis to CPET cart software include: 1) automatic detection of a suitable ramp protocol to achieve an exercise duration of approximately 10 minutes using standard data such as height, weight, age and sex and training status (untrained, moderately-trained, endurance-trained); 2) an automatic VO_2 plateau detection and an automatic calculation of the multi-parameter score based on secondary criteria; 3) calculation of submaximal parameters showing evidence supporting their assessment in HFrEF (OUES, EOV, and VE/VCO_2 slope); 4) an automatic determination of VO_2 off-

kinetics; and 5) a uniform output of the raw data in a consistent Excel or CSV format across all manufacturers to generate a large database, in which all research institutes can easily merge their data regardless of the CPET system used. The technological integration of these features would make the results of this thesis even more applicable and user-friendly. A future step could also be clinical decision trees using VO_{2peak} and submaximal markers to provide support in the clinical interpretation of CPET data and the use of CPET as a tool for differential diagnostic of exercise limitations.

Publication 6 involved a novel approach to combine different health-related physical fitness measures to produce a composite outcome. In the future, physical fitness testing batteries for different settings should be deployed to investigate physical fitness in clinical practice. To prescribe the "polypill" exercise in clinical routine, physical fitness components need to be initially established and then regularly measured, similar to other clinical variables such as blood pressure or blood lipids. Further studies are needed to determine whether large data approaches and composite measures or simple physical fitness testing batteries will play a major role in achieving this goal. Lastly, guidelines on the assessment of physical fitness in health research and clinical application, including an updated definition of physical fitness, are urgently needed to advance the field.

9.5 Conclusions

This Ph.D. project includes a literature review and a cross-sectional investigation (COmPLETE Project) with two parts, COmPLETE-Health and COmPLETE-Heart. COmPLETE-Health examined the physical fitness components and CV properties in a healthy population sample aged between 20 and 90 years. Separately, in COmPLETE-Heart, the same markers in patients at different stages of chronic HF were assessed. The included manuscripts in this thesis cover various aspects of CPET and physical fitness assessment in health and HF from methodological aspects to reference values for clinical application, and to new frontiers in risk stratification using physical fitness parameters.

In summary, the following conclusions can be drawn as a result of the work encompassed in this thesis. The performed literature review evaluating commonly-used ventilatory gas exchange variables for risk stratification and management of HFrEF concluded that, although some CPET variables met more criteria for surrogate endpoints than others, evidence supporting the clinical assessment of variables beyond peak VO_2 is well established. A multi-variable approach is therefore recommended in routine clinical testing and should include VO_{2peak} or percent predicted VO_{2peak}, the VE/VCO_2 slope, and EOV. OUES and $P_{ET}CO_2$ should be considered as additional variables. The application of the new exhaustion criteria for each age group (20–39 years: RER_{max} ≥ 1.13, $APMHR_{210}$ ≥ 96%, $APMHR_{208}$ 93%; 40–59 years: RER_{max} ≥ 1.13, $APMHR_{210}$ ≥ 96%, $APMHR_{208}$ ≥ 93%; 60–69 years: RER_{max} ≥ 1.10, $APMHR_{210}$ ≥ 99%, $APMHR_{208}$ ≥ 89%) reduces the risk of underestimating VO_{2peak} and provides a simple, yet effective tool for healthy individuals undergoing CPET. The multi-parameter score provides a useful tool for research purposes. Lower values than those suggested increase false-positive results, assuming incorrectly that participants are exhausted. The established reference values for maximal and several submaximal CPET values

over a large age range of a healthy European cohort are novel and differences to other studies are clinically highly relevant. Vigorous-intensity PA showed a strong positive association with higher VO_{2peak} and other performance-related CPET parameters within the healthy cohort, supporting the implementation of higher intensity physical exercise in health promotion. Looking beyond the traditional CPET markers, VO_2-kinetics can provide an acceptable substitute if VO_{2peak} cannot be determined. VO_2 off-kinetics appears to be superior for distinguishing patients with HF and healthy participants compared to VO_2 on-kinetics and ramp-kinetics. Finally, when combining several physical fitness biomarkers, a significant difference in HD between healthy individuals and patients with HF was observed. The application of HD could strengthen a comprehensive assessment of physical fitness and may present an optimal target for interventions to slow the decline of physical fitness with aging and, therefore, to increase healthspan.

Overall, this thesis was able to fill several research gaps within the scientific literature, as well as exploring new parameters and approaches of measuring physical fitness markers as clinical vital signs over the lifespan of both healthy individuals and those chronically ill with HF. This work has contributed a puzzle piece to research and initiatives needed to enable early prevention of chronic disease, increase health span and ultimately meet the approaching challenge of the changing age demographic known as “the silver tsunami.”

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