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Improving exercise testing methods and interpretation in human health and diseases

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Improving exercise testing methods and interpretation in human health and diseases

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Editorial: Improving exercise testing methods and interpretation in human health and diseases

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KEYWORDS

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Editorial on the Research Topic
Improving exercise testing methods and interpretation in human health and diseases

Introduction

Exercise testing is a valuable diagnostic tool that is pivotal to successful exercise prescription. Several new testing modalities and outcomes have emerged in the last decades, broadening the applications of exercise testing and indications for regular assessments. However, there is still much to do to improve exercise testing methods, implementation, interpretation, and significance in specific clinical contexts. The present research topic followed a multimodal approach to exercise testing without any restrictions regarding populations, reflecting the editors' aspiration to highlight the complementary of different exercise tools and outcomes for a wide range of clinical applications. This research topic includes 17 peer-reviewed articles covering various exercise methods (from local muscle testing to maximal whole-body exercise), populations and diseases, including healthy children and adults with various fitness levels and several chronic diseases and pathological conditions: cystic fibrosis, chronic obstructive pulmonary disease (COPD), lung cancer resection candidates, coronary artery disease, individuals with left ventricular assist devices, chronic kidney disease, obesity and bariatric surgery candidates.

Incremental exercise tests: Useful clinical information

Incremental exercise tests with gas exchange measurements objectively assess the integrative functioning of the respiratory, cardiovascular, and muscular systems. These tests are useful to detect physiological adaptations and abnormalities, providing avenues for optimizing exercise prescription. Youxiang et al. found different exercise metabolic responses during incremental treadmill tests between obese children and adolescents with no insulin resistance and those with insulin resistance, with for instance, a lower maximal fat oxidation intensity for the latter, which should be considered when designing exercise interventions targeting fat loss. Tomlinson et al. retrospectively analysed cardiopulmonary exercise testing (CPET) of people with cystic fibrosis of differing glycemic status. They found that people with cystic fibrosis-related diabetes (CFRD) had reduced peak oxygen uptake ($\dot{V}O_{2peak}$) compared to non-CFRD counterparts, which was linked to poorer lung function. They also found that lung function and aerobic capacity differed in direction and magnitude of longitudinal changes, suggesting that $\dot{V}O_{2peak}$ should be considered an independent clinical marker in people with CF with varying glycemic status.

CPET-derived outcomes also have a good prognostic value in various clinical populations. For instance, $\dot{V}O_{2peak}$ can predict mortality and hospitalization in cardiac and respiratory diseases (Arena et al., 2004; Hebestreit et al., 2019). However, such predictive capacity remains to be established in some disorders and specific subgroups of patients, notably for predicting postoperative complications after cardiopulmonary surgery. For instance, while $\dot{V}O_2$ thresholds have proven utility in stratifying postoperative risk following lung cancer resection, they were primarily derived from patients who underwent thoracotomy (Brunelli et al., 2009). Chouinard et al. found that $\dot{V}O_{2peak}$ was also an independent predictor of postoperative morbidity and mortality after minimally invasive video-assisted thoracoscopic surgery for lung cancer resection. However, its ability to discriminate patients with or without adverse outcomes was limited.

CPET is also an important tool for athletes. For example, it might be helpful to determine how the performance of the cardiorespiratory system in athletes could be affected during periods of training reduction or cessation (e.g., off-season, near-competition, post-injury/surgery period). However, performing a maximal CPET might not always be advisable in these circumstances. With this framework in mind, Oyarzo-Aravena et al. sought to identify the determinants of the cardiorespiratory optimal point, corresponding to the lowest minute ventilation to $\dot{V}O_2$ ratio obtained during a CPET in endurance athletes. Using principal component analysis, they found that this submaximal parameter was closely related to the second ventilatory threshold and $\dot{V}O_{2peak}$, supporting its utility to track physiological performance in the aforementioned circumstances, where a submaximal assessment might be preferred.

Predicting $\dot{V}O_{2peak}$ might also be of value in athletes for better interpretation of the test or when indirect calorimetry is unavailable. However, some predictive equations might be misleading if they are utilized in populations that differ from those selected to develop the prediction model. For instance, some physiological impairments may be masked in athletes if their performances are carelessly compared to predictive values derived from non-athletic populations. Jurov et al. compared the $\dot{V}O_{2peak}$ measured in 580 competitive cyclists to $\dot{V}O_{2peak}$ estimated from four predictive equations and found that the FRIEND (Fitness Registry and the Importance of Exercise National Database) equation was the most suitable for predicting $\dot{V}O_{2peak}$ in this athletic population.

Incremental exercise tests: New procedures and outcomes for intensity distribution

CPET-derived outcomes like ventilatory or lactate thresholds are also relevant to set training intensities. However, the need for special equipment and operators has stimulated research to develop new methods to detect exercise intensity zones. Rogers and Gronwald discussed in a narrative review the utility of the short-term scaling exponent alpha1 of Detrended Fluctuation Analysis (DFA a1), an HRV index based on fractal correlation properties, to delineate exercise intensity domains. Discrete numerical values of this indicator have shown great associations with physiological thresholds in both athletic and clinical populations. Another promising application of DFA a1 is the management of internal load for specific exercise modalities, which have proven efficacy in improving health markers in several populations but for which assessing intensity distribution is not straightforward [e.g., eccentric cycling (Barreto et al., 2021)].

Several studies have evaluated the ability of various versions of the Talk Test to discriminate exercise intensities, intending to make exercise prescription even more simple and accessible. For instance, as the ability to vocalize is becoming more difficult with increasing ventilatory requirements, some studies found a good approximation of the ventilatory threshold from these Talk Tests (Persinger et al., 2004). To provide a more standardized form of the Talk Test, Mahmod et al., developed the time-controlled monosyllabic Talk Test. They found that this version was more suited to detect the intensity of light, moderate, and vigorous exercise intensity, as compared to self-paced Counting Talk Test, offering new avenues in the field of exercise prescription.

Six-minute walk test

While the previous sections supported the clinical significance of CPET, the value of alternative exercise tests should not be discarded. Field exercise tests can be used in settings where access to CPET is limited and may provide complementary information and be better suited for regular assessments. The 6-min walk test (6MWT) is among the most popular field exercise tests. While it provides only limited information on the causes of exercise limitation, the 6MWT remains widely used as a simple low-cost alternative for measuring functional exercise capacity in a wide range of clinical populations. This point is illustrated by two systematic reviews published in this

research topic that benefited from the fact that this test is often selected as a clinical trial endpoint to discuss the efficacy of exercise interventions in specific clinical contexts based on changes in 6MWT performance. Song et al. used changes in 6MWT as a surrogate of training efficacy in hemodialysis patients and found that the combination of aerobic plus resistance exercise was the most effective intervention to improve 6MWT in this population. In the context of bariatric surgery, Jabbour et al. discussed the importance of enhancing the preoperative fitness levels of patients to improve postoperative outcomes. In this context, this review highlights the clinical significance of the 6MWT, which is the most used exercise test and that has proven to be highly feasible and sensitive to detect functional improvements in bariatric surgery candidates.

It is also important to interpret the performance of the 6MWT for exercise counselling, and performance is often compared to a predicted performance from a reference equation. However, some equations obtained from a healthy population may not be adapted for interpreting performance in specific clinical populations. Lenasi et al. demonstrated that the widely used Enright-Sherill equation leaded to a prediction that was 52 m lower than the actual performance of people with stable coronary artery disease, pointing out the importance of developing populations-specific prediction equations.

Although the 6MWT is a simple and well-tolerated test in several clinical populations, it still has the inconvenience of requiring a quiet corridor of at least 30 m, which might be a limitation for its implementation in some hospital settings. The 6-min step test (6MST), a portable test that only requires a stepper, can thus be an interesting alternative. The clinimetric properties of the 6MST have been established in various populations, including stable people with COPD (Borel et al., 2010). Ribeiro et al. extended its use to hospitalized COPD patients with an acute exacerbation by confirming its concurrent validity, offering a new simple alternative to guide and monitor therapeutic strategies in these fragile patients.

Local peripheral muscle testing

Muscle exercise testing encompasses various components, i.e., strength, power, endurance and fatigability. Altered muscle function may negatively impact various activities of daily living and seems predictive for various health issues, such as sports injuries or falls. Muscle exercise testing allows to identify specific muscle abnormalities in a given population and having reliable and accessible tests and outcomes will help to guide therapeutic interventions and assess their effectiveness. For instance, using isometric and isokinetic testing of several muscle groups, Gobbo et al. reported large impairments in muscle strength in people with left ventricular assist devices, which were not correlated with $\dot{V}O_{2peak}$. While these results offer a rationale for implementing resistance training modalities in this population, they also suggest that increasing muscle strength may not be sufficient to translate into improved aerobic endurance performance. Muscle testing may also allow to detect functional differences between populations and shed light on the underlying mechanisms. For instance, the meta-analysis conducted by Souron et al. showed that the differences in muscle fatigability and endurance between healthy children and adults were dependent on the exercise testing modality (i.e., isometric vs. dynamic exercise tests).

Vertical jump testing is another form of muscle testing, which offers simple markers of neuromuscular function that are relevant to daily life activities. Jump tests can be performed safely even in old individuals (Buehring et al., 2015) and jump height can be reliably estimated with a force plate, which is considered the gold-standard method. Nevertheless, as this latter is not always easily accessible, Gruber et al. sought to assess the validity and reliability of counter movement jump height using a sport watch (Polar Vantage V2). Their positive results support the use of this technology to reliably monitor jump height outside the lab, fostering the implementation of jump testing in clinical settings.

Muscle testing may also be useful in the context of injury prevention, for instance, in alpine skiers by detecting potential muscle imbalances derived from the hamstrings-to-quadriceps strength ratio (Spörri et al., 2017). However, such outcomes are often not sufficient and it might be useful to complement these functional measures with muscle architecture assessments. This is supported by the findings of Fitze et al., who found that average anatomical cross-sectional area of the biceps femoris long head measured by ultrasound was associated with the occurrence of traumatic lower extremity injuries in youth skiers. The cost and low portability of high-end ultrasonography devices is however a limitation to their implementation in sports and clinical settings. Ritsche et al. demonstrated that lower limb muscle architecture measurements like muscle thickness could be reliably assessed with handheld portable ultrasound system with good agreement with a high-end laboratory device, fostering the possibility of developing new screening methods and algorithms in the context of injury prevention based on the combination of affordable muscle architecture and functional assessments.

Assessment of exercise-induced gastrointestinal perturbations

Exercise testing can also be helpful in identifying markers or predictors of syndromes associated with strenuous/prolonged exercises and/or performed under environmental stress. For instance, various hypoxic exercise tests have been developed to identify physiological predictors of severe high-altitude illness (Richalet et al., 2012). Some exercise modalities can also be used to study exercise-induced gastrointestinal and systemic disturbances (Costa et al., 2022). Young et al. demonstrated that a 2 h high-intensity interval exercise was sufficient for inducing gastrointestinal and systemic disturbances, with variable reliability (from poor to excellent) of several usual biomarkers. These data support the need to assess a cluster of biomarkers and interpret them collectively to determine the incidence and severity of exercise-induced gastrointestinal syndrome.

Conclusion

The collective publications in this research topic support exercise testing as a versatile tool providing relevant information in virtually all populations, irrespective of age, disease, and severity. Such studies are pivotal in fostering the implementation of exercise testing in clinical practice and guiding the choice among all the existing testing procedures.

Author contributions

All authors contributed to writing this editorial and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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The Effect of Dysglycaemia on Changes in Pulmonary and Aerobic Function in Cystic Fibrosis

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Cross-sectional studies have reported lower pulmonary and aerobic function during exercise in people with cystic fibrosis-related diabetes (CFRD) compared to non-CFRD counterparts. However, this association has yet to be longitudinally investigated. Therefore, this study examines these differences over time between people with cystic fibrosis (CF) of differing glycaemic status. Annual review data, including cardiopulmonary exercise tests and pulmonary function tests, were retrospectively analysed at baseline (T0, *n* = 82) and at a one-year follow-up (T1, *n* = 54). Data was analysed in three groups: normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and CFRD. Further analyses were undertaken, with a dichotomous split of NGT and a combined IGT/CFRD group. At baseline, a significant reduction in the majority of variables, including forced expiratory volume in one second (FEV_1) and maximal oxygen uptake (VO_{2max}), was observed in the CFRD (*n* = 19) group compared to NGT (*n* = 58). At follow-up, no significant differences were observed, and no interaction effect between CFRD status and time was identified. FEV_1 and VO_{2max} presented with varying directions and magnitudes of change within patients. In summary, patients with CFRD have a reduced aerobic and pulmonary function compared to non-CFRD counterparts, although such changes disappeared at follow up. Varying responses for FEV_1 and VO_{2max} highlight the need to consider both variables as independent markers of function in CF.

Keywords: cystic fibrosis related diabetes, cardiorespiratory fitness, oxygen uptake, longitudinal data, pulmonary disease

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INTRODUCTION

Cystic fibrosis (CF), the most common autosomal recessive condition in the Caucasian population, affecting ~10,500 people in the United Kingdom (UK) (Cystic Fibrosis Trust, 2019). Due to increased life expectancy, non-respiratory co-morbidities are becoming more significant contributors to ill-health and prognosis (Hebestreit et al., 2019; Keogh et al., 2019).

Abbreviations: ANCOVA: analysis of covariance; ANOVA: analysis of variance; BMI: body mass index; CF: cystic fibrosis; CFRD: cystic fibrosis related diabetes; CPET: cardiopulmonary exercise testing; ES: effect size; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; GET: gas exchange threshold; HR_{max}: maximal heart rate; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; PI: pancreatic insufficiency; PPO: peak power output; RER: respiratory exchange ratio; S_{max}: supramaximal verification testing; T0: baseline data; T1: one-year follow up data; UK: United Kingdom; V_{Epeak}: peak minute ventilation; V_E/VCO_2: ventilatory equivalent of carbon dioxide; VO_{2max}: maximal oxygen uptake; VO_{2peak}: peak oxygen uptake.

Estimates show that 85% of people with CF have a degree of pancreatic insufficiency (PI) (Singh and Schwarzenberg, 2017), and in 2018, 30% of people over 10 years of age were undergoing treatment for CF-related diabetes (CFRD) in the UK (Cystic Fibrosis Trust, 2019). It is believed incidence of CFRD rises with age, with increasing reported prevalence from 11 to 24% over 5 years (Lanng et al., 1995). Given that the pancreas is one of the earliest affected organs in CF (Gibson-Corley et al., 2016) and insufficiency is becoming a major determinant of morbidity and mortality (Causer et al., 2020), the number of people being screened for CFRD has increased from 42% in 2004 to 81% in 2018 (Ukcf Database, 2006; Cystic Fibrosis Trust, 2019). As prevention of CFRD is cited as a research priority within the CF community (Rowbotham et al., 2018), there is a necessity for research that explore factors associated with CFRD.

Several studies indicate that glucose intolerance is associated with poor clinical function (Ziegler et al., 2011; Foster et al., 2018; Causer et al., 2020), although some report contradicting results in relation to CFRD and lung function. For example, forced expired volume in one second (FEV_1) and forced vital capacity (FVC) have been reported to be reduced in CFRD (Koch et al., 2001; Causer et al., 2020), but a lack of difference (relative to those with normal glucose tolerance, NGT) is reported elsewhere (Ziegler et al., 2011; Foster et al., 2018). Given the variability in the relationship between CFRD and FEV_1, alternative factors must be investigated to create a detailed and holistic clinical profile for patients.

With cardiopulmonary exercise testing (CPET) acknowledged as the gold-standard assessment for determining maximal oxygen uptake (Hebestreit et al., 2015), it has been established that higher levels of aerobic fitness (represented by peak/maximal oxygen uptake, $VO_{2peak/max}$) are important in CF. Higher levels of aerobic fitness are associated with reduced risk of hospitalisation and better prognosis (Pérez et al., 2014; Hebestreit et al., 2019). To date, minimal research exists on how CFRD affects aerobic fitness, although a single cross-sectional study established that people with CFRD have reduced aerobic fitness compared to non-CFRD counterparts (Causer et al., 2020). When considering longitudinal designs, research has assessed temporal interactions between glycaemic status, aerobic fitness, and physical activity, finding that CFRD negatively impacts changes in FEV_1 (Schneiderman et al., 2014). However, this latter study did not explicitly identify the association between glycaemic status and aerobic fitness over time. Therefore, this lack of data provides the rationale for the present analysis.

This study sought to investigate differences in pulmonary and aerobic function in people with CF of differing glycaemic status, generating novel longitudinal data on the relationship between these factors, whilst providing robust replication of previous cross-sectional analyses (Causer et al., 2020).

MATERIALS AND METHODS

Study Design and Ethics

A retrospective analysis of clinical data from the Royal Devon and Exeter Cystic Fibrosis Centre, collected between 2015 and 2018, was performed. As per national guidelines (National Institute for Health and Care Excellence, 2017), data collected at annual review includes pulmonary function testing, nutritional and diabetic review, and exercise testing. This data is recorded *via* a standardised *proforma* (Cystic Fibrosis Trust, 2021) for use by the national registry, with study data collated using this platform.

As this study analysed retrospective, routinely collated data, and was anonymised prior to analysis, full ethical review and patient consent was not required. Approval for use of anonymised data was obtained from the Health Research Authority (IRAS 238996).

Participants and Timeline

All patients included in this assessment had confirmed diagnosis of CF based on clinical features, elevated sweat chloride (>60 mmol L^{-1}), and genotyping where possible. The CFTR2 database (Cystic Fibrosis Foundation, 2011) assisted with genotype classification, with Class I/II mutations regarded as "severe."

Data were analysed from two time-points: baseline (T0) and at one-year follow-up (T1). Each patient's baseline measure was not necessarily in the same year as one another, but represented the first year they performed CPET at annual review. Regardless of T0 date, T1 measures were one-year later for all patients.

Anthropometry and Pulmonary Function

Body mass and stature were measured to the nearest 0.1 kg and 0.01 m, respectively, with body mass index subsequently calculated. Pulmonary function (FEV_1 and FVC) was assessed *via* flow-volume loop spirometry, with results recorded as absolute values and as percent of predicted ($\%_{Pred}$), using normative values from the Global Lung Initiative (Quanjer et al., 2012).

Glycaemic Status

Through oral glucose tolerance testing and continuous glucose monitoring, both of which are utilised in CF management (Cystic Fibrosis Trust, 2004), participants were categorised into NGT (<7.8 mmol L^{-1}), impaired glucose tolerance (IGT; 7.8–11.0 mmol L^{-1}), and CFRD (≥ 11.1 mmol L^{-1}). These boundaries are in line with existing guidelines (American Diabetes Association, 1997) and have been adopted by previous CFRD studies to assess exercise (Causer et al., 2020). Glycaemic status data was obtained from annual review *proforma* (Cystic Fibrosis Trust, 2021).

Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing was performed *via* cycle ergometry (Lode Excalibur; Lode, Groningen, Netherlands). Breath-by-breath pulmonary gas exchange using a metabolic cart (Metalyzer II; Cortex Biophysik, Leipzig, Germany) determined VO_{2peak} which was subsequently confirmed as a maximal VO_2 (VO_{2max}) by supra-maximal verification (S_{max}) at 110% peak power output (PPO) of that achieved in an initial ramp-incremental test (10–30 W min^{-1}) to volitional exhaustion; a process validated in both adults and children with CF (Saynor et al., 2013; Causer et al., 2018). If VO_{2max} could not be verified

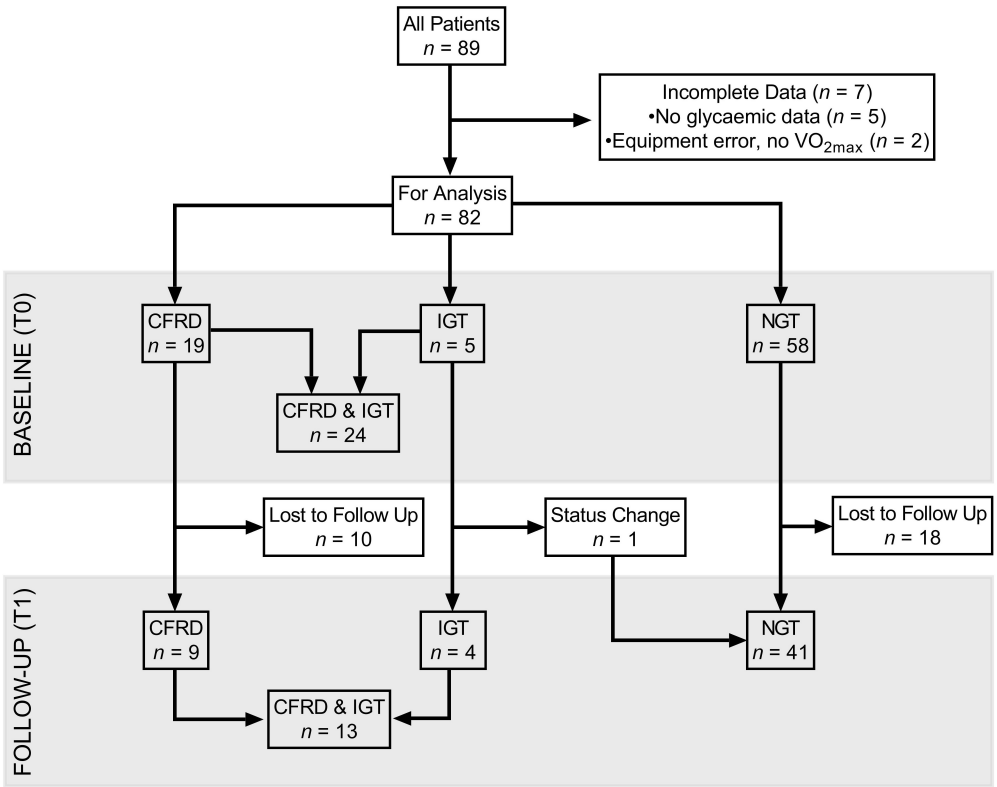

FIGURE 1 | Flow diagram, characterising follow-up and drop-out of participants, split by glycaemic status in three groups (CFRD, IGT, NGT), and when considered dichotomously (IGT/CFRD, NGT) at baseline and one-year follow-up. CFRD: cystic fibrosis related diabetes; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; T0: baseline measurement; T1: 1 year follow-up; VO_{2max}: maximal oxygen uptake.

via S_{max} testing, secondary criteria were utilised to ascertain maximal efforts (Radtke et al., 2019).

All CPET derived variables were interpolated to 10 s averages, with highest values taken as the peak was achieved. A slope of ventilatory equivalents of CO_2 (V_E/VCO_2) was established from the start of the ramp test to the respiratory compensation point. The gas exchange threshold (GET) was calculated using the V-slope method (Beaver et al., 1986), and corroborated using ventilatory equivalents of CO_2 and O_2.

Data Analysis

Firstly, for cross-sectional analyses at baseline and follow-up, one-way analyses of variance (ANOVA) with *post hoc* Bonferroni-corrected independent-sample *t*-tests identified main effects of a group upon variables. In addition, with specific relation to VO_{2max} (mL kg^{-1} min^{-1}) and analysis of covariance (ANCOVA), controlling for FEV_1 ($\%_{Pred}$) was undertaken at both time-points to identify effect of group, independent of lung function. Pearson's correlation coefficients established relationships between FEV_1 and VO_{2max}.

Secondly, to identify changes over time, a mixed-model ANOVA determined interaction effects between groups and time for FEV_1 and VO_{2max}, from T0 to T1. This mixed-model ANOVA used dichotomous groups, whereby IGT patients were included within the CFRD group (due to having a degree of PI) to counter reduced statistical power due to loss of follow-up data. *Post hoc* Bonferroni-corrected paired-sample *t*-tests highlighted significant differences between variables at differing time-points. Additionally, mixed-model ANCOVAs were specifically performed for VO_{2max} (mL kg^{-1} min^{-1}), controlling for FEV_1 ($\%_{Pred}$) and age (years). Chi-square tests and logistic regression identified associations between direction of change over time and glycaemic status, along with probability of any increase/decrease in either FEV_1 or VO_{2max}. Pearson's correlation coefficients established relationships between changes in both FEV_1 and VO_{2max}.

Data are expressed as means (± standard deviation). Effect sizes (*ES*) were expressed using thresholds of Cohen's *d* describe differences between groups (small $0.2 < 0.5$, medium $0.5 < 0.8$, large ≥ 0.8) and correlation coefficients (small $0.1 < 0.3$, medium $0.3 < 0.5$, large ≥ 0.5) (Cohen, 1992). Analyses were performed using SPSS v.26 (IBM; Armonk, NY, United States), with $p < 0.05$ indicating statistical significance.

RESULTS

Patient Inclusion and Follow-Up

Records identified $n = 89$ patients who had undergone CPET within the specified timeframe and were eligible for inclusion.

However, due to incomplete clinical datasets, n = 82 were included in analyses at T0 [paediatric (<18 years), n = 31]. Of the n = 7 excluded at T0, n = 2 were removed due to equipment errors during CPET, resulting in failure to obtain accurate VO_{2max} (primary exercise variable), and n = 5 due to lack of information on glycaemic status (**Figure 1**). The characteristics of included patients at T0 and T1 are shown in **Tables 1**, **2**, respectively.

At T1, n = 54 (paediatric n = 17) patients were included. Of those lost to follow-up (i.e., no CPET at T1), 35% in the NGT and 82% in the IGT/CFRD group were identified as having severe mutations. A breakdown of genotypes is provided in **Supplementary Material**.

Validity of Cardiopulmonary Exercise Testing

At T0, 40% of participants did not have VO_{2max} verified *via* S_{max} tests (n = 11 did not perform S_{max}, n = 22 not verified despite undergoing S_{max}), although all satisfied secondary criteria were used to verify maximal efforts. At T1, 31% did not have a verified VO_{2max} (n = 2 did not perform S_{max}, n = 15 not verified), although all patients satisfied secondary criteria. Consequently, as all participants satisfied criteria for maximal efforts, the term "VO_{2max}" is used herein.

Moreover, within **Tables 1**, **2**, a number of variables are unavailable due to equipment malfunctions during CPET (HR_{max}), accidental omission of data recording during testing (PPO), and non-detection of GET. However, despite some missing data, patients have been continued forward for analyses as they still possessed data pertaining to VO_{2max} – the primary exercise variable of interest.

Finally, analyses revealed that allometric scaling (Armstrong and Welsman, 1994) was not required for VO_{2max} as no significant correlation was identified between body mass and ratio-scaled VO_{2max} ($r = -0.19$, $p = 0.09$).

Baseline Data

One-way ANOVA showed a significant main effect of group in the majority of pulmonary and exercise-related variables, including FEV_1 and VO_{2max} (**Table 1**). Predominantly, these significant results were driven by differences between NGT and CFRD, apart from V_E/VCO_2 – driven by differences between NGT and IGT. When ANCOVA was undertaken for VO_{2max} (controlling for FEV_1), the main effect of group disappeared ($p = 0.25$).

In assessing combined groups, *post hoc* analyses from the mixed design ANOVA revealed significantly higher FEV_1 within the NGT group relative to the combined IGT/CFRD group at baseline (80.90 ± 20.50 vs. 67.11 ± 20.41%$_{Pred}$, p = 0.035, ES = 0.67, **Figure 2**). Furthermore, a significantly higher VO_{2max} was identified in the NGT group relative to the combined IGT/CFRD group (30.37 ± 7.80 vs. 25.38 ± 4.94 ml kg^{-1} min^{-1}, p = 0.030, ES = 0.69, **Figure 2**). However, when FEV_1 was controlled for in a mixed-model ANCOVA, this became non-significant (p = 0.14), whereas controlling for age ensures that significance between groups is maintained (p = 0.013).

The correlation between FEV_1 and VO_{2max} was medium and statistically significant for the whole group (r = 0.44, $p < 0.001$), and the NGT group (r = 0.38, p = 0.003). A medium, non-significant coefficient was present in the IGT/CFRD group (r = 0.33, p = 0.12).

One-Year Follow-Up

One-way ANOVA showed no main effect of group for any variable (**Table 2**). Moreover, for VO_{2max}, one-way ANCOVA identified no significant effect of group (p = 0.17).

When assessing data using combined groups, no significant difference was evident for FEV_1, although a medium effect size remained (NGT = 78.47 ± 18.50, IGT/CFRD = 67.20 ± 21.24%$_{Pred}$, p = 0.06, ES = 0.59). A higher VO_{2max} was observed in the NGT group relative to the combined IGT/CFRD group (30.21 ± 7.31 vs. 25.20 ± 4.14 ml kg^{-1} min^{-1}, p = 0.019, ES = 0.75, **Figure 2**), although this significance disappeared when controlling for FEV_1 in ANCOVA models (p = 0.13) but remained when age was controlled for (p = 0.005).

The correlation between FEV_1 and VO_{2max} was medium and statistically significant for the whole group (r = 0.44, p = 0.001) and the NGT group (r = 0.48, p = 0.001), but not the IGT/CFRD group (r = 0.12, p = 0.59).

Longitudinal Changes

One patient (adolescent male) changed from IGT to NGT between T0 and T1 (**Figure 1**). All other patients remained stable with regards to glycaemic status.

No significant difference was identified for FEV_1 over time in the combined IGT/CFRD group (67.11 ± 20.41 vs. 67.20 ± 21.24%$_{Pred}$, p = 0.97, ES = 0.00), whereas a near-significant difference was identified within the NGT group (80.90 ± 20.50 vs. 78.47 ± 18.50%$_{Pred}$, p = 0.051, ES = 0.12). No significant differences were observed with regards to VO_{2max} in either the NGT (30.37 ± 7.80 vs. 30.21 ± 7.31 ml kg^{-1} min^{-1}, p = 0.83, ES = 0.02) or combined IGT/CFRD group (25.38 ± 4.94 vs. 25.20 ± 4.14 ml kg^{-1} min^{-1}, p = 0.90, ES = 0.04).

Within the NGT group, FEV_1 and VO_{2max} increased in 35 and 50% of patients, respectively. Within the IGT/CFRD group, FEV_1 and VO_{2max} similarly increased by 36 and 50%, respectively. Association between glycaemic status and direction of change resulted in non-significant Chi-square tests for both FEV_1 ($\chi^2 < 0.01$, p = 0.96) and VO_{2max} ($\chi^2 < 0.01$, p = 1.00) and non-significant logistic regressions for both FEV_1 (β = 1.03, p = 0.96) and VO_{2max} (β = 1.00, p = 1.00).

The correlation between the change in FEV_1 and VO_{2max} was small but not statistically significant at the whole group level (r = 0.20, p = 0.14, **Figure 3**), within the NGT (r = 0.27, p = 0.09) or IGT/CFRD groups (r = 0.07, p = 0.80).

DISCUSSION

This is the first study to longitudinally examine how dysglycaemia impacts upon pulmonary and aerobic function in patients with CF. Three major findings are reported: (1) at baseline, patients with CFRD had statistically lower function in most

TABLE 1 | Participant characteristics including anthropometry, pulmonary function, and exercise-based outcomes at baseline (T0).

	NGT (*n* = 58)	IGT (*n* = 5)	CFRD (*n* = 19)	*P*-value
Participants				
Males (%)	64	60	42	–
Age (y)	25.8 ± 14.5	22.2 ± 8.0	27.0 ± 10.5	0.78
ΔF508 Status* (*n*)	18/35/5	4/1/0	7/10/2	–
Pancreatic Insufficient (*n*)	34	5	19	–
Anthropometry				
Stature (m)	1.65 ± 0.13	1.66 ± 0.12	1.64 ± 0.8	0.93
Body mass (kg)	65.3 ± 17.9	60.0 ± 11.3	59.5 ± 11.4	0.36
BMI (kg m^{-2})	23.42 ± 4.15	21.87 ± 3.21	21.94 ± 2.80	0.27
Spirometry				
FVC (L)	3.76 ± 1.19	3.45 ± 0.95	3.06 ± 0.85	0.06
FVC ($\%_{Pred}$)	90.8 ± 13.7	81.4 ± 14.4	76.1 ± 19.9	**<0.01**[a]
FEV_1 (L)	2.83 ± 1.03	2.62 ± 0.73	1.97 ± 0.83	**0.01**[a]
FEV_1 ($\%_{Pred}$)	81.6 ± 18.9	72.8 ± 12.6	58.2 ± 23.9	**<0.01**[a]
Exercise				
PPO (W)	172 ± 63†	149 ± 61	126 ± 52	**0.02**[a]
PPO (W kg^{-1})	2.68 ± 0.75†	2.41 ± 0.56	2.11 ± 0.74	**0.02**[a]
VO_{2max} (L min^{-1})	1.88 ± 0.65	1.44 ± 0.44	1.41 ± 0.44	**<0.01**[a]
VO_{2max} (mL kg^{-1} min^{-1})	29.44 ± 8.30	23.66 ± 2.75	23.69 ± 5.93	**0.01**[a]
HR_{peak} (beats min^{-1})	175 ± 18†	181 ± 11†	161 ± 13†	**0.03**[a]
GET (mL kg^{-1} min^{-1})	17.24 ± 4.81†	14.99 ± 2.38	13.77 ± 3.36†	**0.02**[a]
GET ($\%VO_{2max}$)	59.3 ± 10.7†	63.3 ± 7.3	57.4 ± 10.5†	0.54
V_{Epeak} (L min^{-1})	92.47 ± 37.24	94.38 ± 30.13	68.62 ± 22.54	**0.03**[a]
RER	1.58 ± 0.34	1.87 ± 0.23	1.49 ± 0.27	0.06
V_E/VCO_2	35.4 ± 5.1	44.5 ± 5.1	34.2 ± 4.7	**<0.01**[b]

*Data are expressed as means ± standard deviation. *ΔF508 status provided as homozygous/heterozygous/none. Text in bold demonstrates a significant main effect of group, obtained from one-way ANOVA ($p < 0.05$), driven by significant post hoc ($p < 0.05$) between normal glucose tolerance (NGT) and cystic fibrosis-related diabetes (CFRD) (a) or NGT and impaired glucose tolerance (IGT) (b). Incomplete data due to aforementioned reasons at T0 (†): peak power output (PPO; NGT, n = 56); peak heartrate (HR_{peak}; NGT, n = 41; IGT, n = 4; CFRD, n = 13); gas exchange threshold (GET; NGT, n = 57; CFRD, n = 16). BMI: body mass index, FVC: forced vital capacity, FEV_1: forced expiratory volume in 1 s, VO_{2max}: maximal oxygen uptake, HR_{peak}: peak heart rate, GET: gas exchange threshold, PPO: peak power output, RER: respiratory exchange ratio, V_{Epeak}: peak minute ventilation, V_E/VCO_2: ventilatory equivalent for carbon dioxide, NGT: Normal glucose tolerance, IGT: Impaired glucose tolerance, CFRD: cystic fibrosis related diabetes.*

pulmonary and aerobic function measures; (2) at follow-up, no statistically significant differences were found between groups for any measure; although when dichotomously grouped, statistically lower values were found for VO_{2max} within the CFRD/IGT group; and (3) VO_{2max} and FEV_1 presented with contrasting magnitude and direction of change over time.

Previous studies have established associations between poorer glycaemic status and reduced pulmonary and aerobic function (Lanng et al., 1995; Koch et al., 2001; Foster et al., 2018), although this finding is inconsistent (Ziegler et al., 2011) and alternative indices of health status should be considered (i.e., VO_{2max}). Previous cross-sectional exercise-oriented research from Causer et al. (2020) found no significant differences between groups of differing glycaemic status for parameters of PPO, HR_{peak}, V_{Epeak}, and V_E/VCO_2, conflicting with our findings. However, VO_{2max} within the work of Causer et al. (2020) was higher in the NGT group than the present study, and V_E/VCO_2 – which is linked to decreased pulmonary perfusion (Arena et al., 2004) – was also higher in the present work. Therefore, reduced VO_{2max} in the current group and a difference in sample sizes between studies likely explains the observed differences. Additionally, Causer et al. (2020) identified lower VO_{2max} in those with PI compared to their non-CFRD counterparts, which is in accordance with the present study.

The novelty of the present study is that it provides longitudinal data on both pulmonary and exercise function, with no significant differences found between groups at follow-up. However, when dichotomously split to create a combined IGT/CFRD group, a non-significant (albeit with medium *ES*) difference was observed for FEV_1, and VO_{2max} was significantly different between groups. Moreover, the further use of ANCOVAs within these analyses indicate that age does not account for the difference in fitness between groups – a useful factor to consider as children will typically have yet to develop CFRD (unlike adult counterparts) and may bias the composition of a NGT groups. However, in contrast, FEV_1 was shown to likely account for differences in fitness between groups. Therefore, further evidencing how disease progression impacts upon multiple parameters and organ systems. In addition, when comparing change over time, whilst no significant group-level differences were observed, approximately 65% of patients presented with declines in FEV_1, whereas 50% declined in

TABLE 2 | Participant characteristics including anthropometry, pulmonary function, and exercise -based outcomes at one-year follow-up (T1).

	NGT (*n* = 41)	IGT (*n* = 4)	CFRD (*n* = 9)	*P*-value
Participants				
Males (%)	71	50	33	–
Age (y)	26.1 ± 12.7	24.7 ± 8.4	24.8 ± 10.4	0.95
ΔF508 Status* (*n*)	17/19/5	3/1/0	4/4/1	–
Pancreatic Insufficient (*n*)	25	4	8	–
Anthropometry				
Stature (m)	1.68 ± 0.12	1.61 ± 0.13	1.63 ± 0.09	0.37
Body mass (kg)	67.3 ± 16.2	59.4 ± 13.9	62.1 ± 15.6	0.48
BMI (kg m^{-2})	23.48 ± 3.94	22.46 ± 2.05	22.89 ± 3.30	0.82
Spirometry				
FVC (L)	3.94 ± 1.11	3.68 ± 1.32	3.28 ± 0.81	0.25
FVC ($\%_{Pred}$)	88.8 ± 12.0	91.0 ± 18.9	83.9 ± 20.1	0.58
FEV_1 (L)	2.85 ± 0.99	2.71 ± 0.73	2.17 ± 0.93	0.17
FEV_1 ($\%_{Pred}$)	77.2 ± 18.6	79.1 ± 6.3	63.9 ± 24.4	0.17
Exercise				
PPO (W)	196 ± 68	154 ± 71	154 ± 59	0.15
PPO (W kg^{-1})	2.92 ± 0.75	2.49 ± 0.63	2.45 ± 0.61	0.15
VO_{2max} (L min^{-1})	2.02 ± 0.63	1.56 ± 0.71	1.58 ± 0.43	0.08
VO_{2max} (mL kg^{-1} min^{-1})	30.38 ± 7.48	25.24 ± 5.85	25.55 ± 3.68	0.09
HR_{peak} (beats min^{-1})	176 ± 26†	181†	181 ± 17†	0.90
GET (mL kg^{-1} min^{-1})	17.46 ± 4.89	13.29 ± 2.09	14.66 ± 4.01†	0.10
GET ($\%VO_{2max}$)	58.1 ± 10.5	53.8 ± 9.6	57.4 ± 10.6†	0.73
V_{Epeak} (L min^{-1})	100.50 ± 34.97	92.24 ± 34.40	83.52 ± 27.40	0.39
RER	1.70 ± 0.38	1.91 ± 0.33	1.78 ± 0.23	0.48
V_E/VCO_2	35.3 ± 5.6	37.8 ± 3.9	35.6 ± 4.9	0.68

*Data are expressed as means ± standard deviation. *ΔF508 status provided as homozygous/heterozygous/none. P-values obtained from one-way ANOVA. Incomplete data due to aforementioned reasons at T1 (†): HR_{peak} (NGT, n = 22; IGT, n = 1; CFRD, n = 6); GET (CFRD, n = 8). BMI: body mass index, FVC: forced vital capacity, FEV_1: forced expiratory volume in 1 s, VO_{2max}: maximal oxygen uptake, HR_{peak}: peak heart rate, GET: gas exchange threshold, PPO: peak power output, RER: respiratory exchange ratio, V_{Epeak}: peak minute ventilation, V_E/VCO_2: ventilatory equivalent for carbon dioxide, NGT: Normal glucose tolerance, IGT: Impaired glucose tolerance, CFRD: cystic fibrosis related diabetes.*

VO_{2max}. The direction and magnitude of individual changes were highly variable (**Figure 3**), whereby such variances may reveal systematic exercise-oriented stability in CF, warranting investigation into whether cardiovascular and/or musculoskeletal function compensates for changing pulmonary function in order to maintain aerobic fitness.

The link between impaired glycaemic status and poorer pulmonary and aerobic capacity has a number of possible explanations. Dysglycaemia triggers oxidative stress and inflammation (Lanng et al., 1995) which can, in turn, induce microvascular dysfunction in the lungs (Totani et al., 2017). Furthermore, diabetes mellitus has an established effect on the immune system in addition to increased levels of glucose in the airways, increasing bacterial proliferation, and placing patients with endocrine PI at greater risk of pulmonary infection, thereby decreasing function. Along with the infection risk, insulin is a potent anabolic hormone, with deficiency promoting catabolism and malnutrition and impairing lung function *via* reduced respiratory muscle mass (Gibson-Corley et al., 2016). Thus, given suggested mechanisms, it is therefore unsurprising that baseline data indicated that patients with CFRD had poorer performance compared to non-CFRD counterparts. Whilst results were expected at baseline in accordance with previous work (Causer et al., 2020), it is surprising to see that differences were not maintained at one-year follow up.

One potential explanation for the lack of significant results at one-year follow up is the loss of patients (n = 82 at T0 vs. n = 54 at T1). This will impact upon statistical power. For example, upon the medium effect size between groups for FEV_1 at T1 that failed to reach statistical significance (ES = 0.59, p = 0.06) or the non-significant difference between time-points for FEV_1 (p = 0.051). Replication of the present study with an increased sample would likely result in statistical significance for these effects.

Moreover, those within the NGT group tended toward milder phenotypes than those with IGT/CFRD, whereas at both time-points, the IGT/CFRD group had a greater proportion of those with severe mutations (Cystic Fibrosis Foundation, 2011) (**Supplementary Material**). A larger percentage of those with severe mutations, and potentially with more severe disease, were lost to follow-up within the IGT/CFRD group. This is supported by the fact that patients in the IGT/CFRD group received more antibiotics than the NGT group over the year (**Supplementary Material**) and may be indicative of disease progression in this group. Subsequently, those that remained at T1 in the IGT/CFRD group may have had

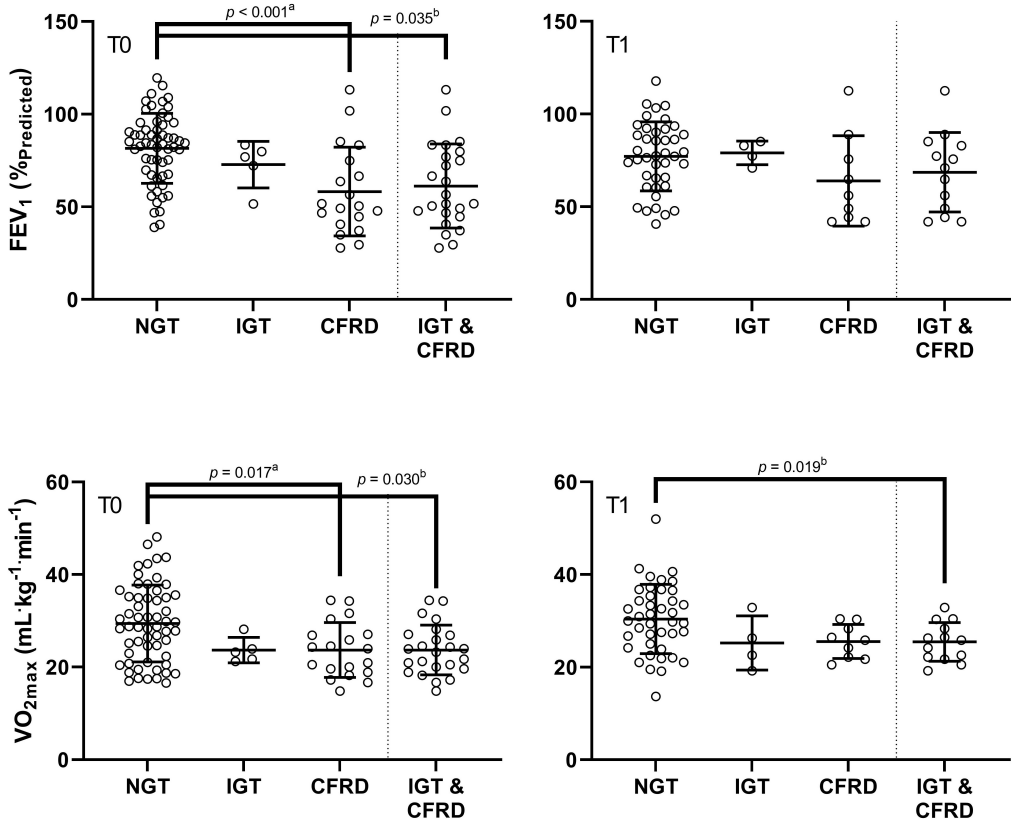

FIGURE 2 | Comparison of FEV_1 ($\%_{Pred}$) and VO_{2max} in groups of differing glycaemic status at baseline (T0) and at one-year follow up (T1). CFRD: cystic fibrosis related diabetes; IGT: impaired glucose tolerance; NGT: normal glucose tolerance. FEV_1: forced expiratory volume in 1 s as a percentage of predicted; VO_{2max}: maximal oxygen uptake. *P*-values are derived from *post hoc* tests following one-way ANOVA (a), and repeated measures ANOVA (b).

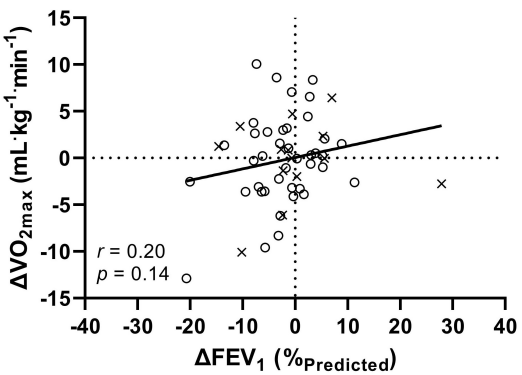

FIGURE 3 | The relationship between the annual change (Δ) in FEV_1 and VO_{2max} for 54 patients with CF. Crosses (×) indicate combined IGT/CFRD group, circles (○) indicate NGT group. FEV_1: forced expiratory volume in 1 s; VO_{2max}: maximal oxygen uptake. All data is presented as T1-T0 (i.e., a negative number indicates a decrease in function over the course of 1 year).

a better pulmonary and aerobic capacity (relative to "lost" IGT/CFRD counterparts), and therefore performed with greater similarity to those in the NGT group. Whilst every endeavour is made to perform annual exercise tests on each patient, this may not always happen. In addition, given that pulmonary function is a predictor of whether patients will undergo annualised CPET (Tomlinson et al., 2020), it is feasible that disease progression has directly affected follow-up results being obtained in this cohort.

There are a number of strengths to this study, including a larger sample size relative to previous studies (Causer et al., 2020) (thus providing increased external validity) and use of gold-standard CPET to ascertain VO_{2max} (Hebestreit et al., 2015). Moreover, analyses were performed on routinely collected data from a single CF centre. Therefore, this group was unlikely to have included heterogeneous treatment regimens that may introduce bias, along with treatments that were occurring prior to the widespread introduction of CFTR modulator therapies, which may impact results. Whilst a strength of the study includes analysis of both children and adults, maturational status should be acknowledged as a potential confounding issue. Insulin resistance increases during puberty, potentially altering CFRD status, although the decline in sensitivity is accompanied by compensatory insulin secretion and recovery after completion of puberty (Kelsey and Zeitler, 2016). This is anecdotally observed within this study, as the only participant to change their glycaemic status was an adolescent male.

In summary, this study found reduced pulmonary function and aerobic fitness in people with CF who also exhibited impaired glycaemic status at a baseline observation, although no significant differences were observed at follow-up. Furthermore, there appeared to be stability in exercise function relative to pulmonary function, thus furthering the evidence for considering VO_{2max} as an independent clinical maker in the assessment and prospective management of CF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Please contact the corresponding author, CW.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

OT: conceptualisation, data acquisition, data analysis, data interpretation, manuscript review, and editing. AS: data analysis, data interpretation, manuscript drafting, review, and editing. LD: data interpretation, manuscript review, and editing. CW: conceptualisation, data analysis, data interpretation, manuscript review, and editing. All authors have approved final manuscript for publication and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.834664/full#supplementary-material

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Regulated Monosyllabic Talk Test vs. Counting Talk Test During Incremental Cardiorespiratory Exercise: Determining the Implications of the Utterance Rate on Exercise Intensity Estimation

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Purpose: When utilizing breathing for speech, the rate and volume of inhalation, as well as the rate of exhalation during the utterance, seem to be largely governed by the speech-controlling system and its requirements with respect to phrasing, loudness, and articulation. However, since the Talk Test represents a non-standardized form of assessment of exercise intensity estimation, this study aimed to compare the utterance rate and the estimated exercise intensity using a newly introduced time-controlled monosyllabic Talk Test (tMTT) versus a self-paced Counting Talk Test (CTT) across incremental exercise stages and examined their associations with the exercise physiological measures.

Methods: Twenty-four participants, 10 males and 14 females (25 ± 4.0 yr; 160 ± 10 cm; 62 ± 14.5 kg) performed two sessions of submaximal cardiorespiratory exercise at incremental heart rate reserve (HRR) stages ranging from 40 to 85% of HRR: one session was performed with a currently available CTT that was affixed to a wall in front of the participants, and the other session was conducted with a tMTT with a 1-s inter-stimulus interval that was displayed from a tablet. In each session, the participants performed six stages of exercise at 40, 50, 60, 70, 80, and 85% HRR on a treadmill and were also asked to rate their perceived exertion based on Borg's 6 to 20 Rating of Perceived Exertion (RPE) at each exercise stage.

Results: The newly designed tMTT significantly delineated all the six stages of incremental exercise ($p \leq 0.017$), while CTT could only delineate exercise stages at 60, 80, and 85% HRR. However, in estimations of exercise intensity, the tMTT demonstrated only moderate associations with HRR and Borg's RPE, similarly to the CTT.

Conclusion: If the purpose of exercise monitoring is to detect the intensity of light, moderate, and vigorous exercise intensity, the tMTT could be more universally applicable. However, due to its larger variability of speech rate across exercise intensities, the time-regulated approach may alter the speech breathing characteristics of the exercising individuals in other ways that should be investigated in future research.

Keywords: cardiorespiratory, exercise intensity, talk test, heart rate, monitoring, prescription

INTRODUCTION

Vocalization or speaking while exercising at one's volition results in competition between the breathing patterns required for linguistic phrasing and the exercising muscles (Baker et al., 2008). Consequently, the ability to vocalize comfortably is compromised when cardiorespiratory exercise intensity exceeds a ventilatory threshold (Quinn and Coons, 2011; Rodríguez-Marroyo et al., 2013). Based on this underlying physiological mechanism, various versions of the "speak while exercise" test, also known as the "Talk Test" (TT), have been introduced as tools to gauge exercise intensity. The evaluation approaches in these tests involve measurement of an individual's reported speaking comfort upon uttering standard text passages at several stages of progressive incremental exercise (Zanettini et al., 2013) as well as calculation of the ratio of the successive counts uttered in a single breath during exercise and those at rest (Norman et al., 2008; Loose et al., 2012).

Utterance production has been found to be strongly associated with pulmonary ventilation and oxygen consumption (Meckel et al., 2002). Moreover, metabolic needs have been shown to significantly outweigh linguistic phrasing at exercise intensities corresponding to 75% of maximal oxygen consumption (Baker et al., 2008) since a high level of ventilatory control is necessary for reasonably normal utterance at higher exercise intensities Hoit et al. (2007). As such, utterance production variables cannot be overlooked in tests that involve vocalization, such as TT, since temporal utterance structure tends to vary even in highly fluent speakers (Zellner, 1994) and may have some effect on outcomes. Additionally, one study investigating the effects of speech production and physiological responses to exercise found that a pre-determined utterance rate of 60–70 words/minute during exercising resulted in a consistent reduction of minute ventilation as exercise intensity increased (Meckel et al., 2002). However, to date, the utterance rate or other utterance characteristics have not been evaluated in any form of TT.

The study aims (1) to compare the utterance rate and the estimated exercise intensity using a newly introduced Talk Test (tMTT) versus a self-paced Counting Talk Test (CTT) across incremental exercise stages and (2) to examine the association between the estimated exercise intensity from the respective TTs and the exercise physiological measures.

MATERIALS AND METHODS

Participants

We included healthy men and non-pregnant women who met the following criteria: the ability to ambulate on a treadmill and no history of cardiovascular disease, significant pulmonary disease, or unstable metabolic disorders. Sample size calculation for correlations was conducted based on a recommended equation (Moinester and Gottfried, 2014). With the number of participants, $n = 25$, and moderate effect size, $d = 0.5$ for bivariate correlation (Sullivan and Feinn, 2012), the statistical power, calculated from the *post hoc* power analysis (Faul et al., 2007) was observed at 0.84. However, only 24 participants (mean age ± standard deviation = 25 ± 4 yr; 10 males) among the university population completed the procedures in this study method and with that number, the statistical power remains the same. The participants were all non-native English speakers and had no reported history of respiratory, speech, or hearing problems. Participants were screened with a Physical Activity Readiness Questionnaire (PAR-Q) as recommended by the American College of Sports Medicine (ACSM). Those who answered "no" to all 7 questions in the PAR-Q were eligible for the exercise testing, and informed consent was obtained from each individual before participation. Participant baseline characteristics are listed in **Table 1**. The study was approved by the Medical Research Ethics Committee, Ministry of Health Malaysia regarding research with humans (NMRR-15-1614-27729).

Design

A cross-sectional study was conducted to compare the utterance rate and estimated exercise intensity across different stages of incremental cardiorespiratory exercise that were conducted in two sessions on two different days within 1 week in the Cardiorespiratory Physiotherapy Laboratory, Universiti Teknologi Malaysia. During the first exercise test session, the participants were required to perform the CTT while exercising on a treadmill (Track Motion; Germany) at incremental heart rate reserve (HRR) stages ranging from 40 to 85% of HRR (Loose et al., 2012). In the second session, an identical exercise protocol was repeated with the tMTT instead of the CTT. The treadmill exercise took about 20 to 30 min only from the whole length of the experiment duration, and the participants were informed earlier to refrain from eating at least 2 h before attending the exercise session.

Methods

During each exercise session, the participants were initially asked to rest in a half-lying position on an adjustable couch while a 12-torso positioned lead electrocardiograph electrodes (Jowett et al., 2005), a pulse oximetry device, and a blood pressure cuff were attached to them. Baseline measurements of the participants' heart rate (HRrest), respiratory rate, oxygen saturation, and

TABLE 1 | Baseline characteristics of the participants.

Characteristics	Values
Age (years)	25 ± 4
Gender	
Male (number, %)	10, 42%
Female (number, %)	14, 58%
Weight (kg) (mean ± SD)	62 ± 14.5
Height (m)	1.6 ± 0.1
Body mass index (kgm^{-2}) (mean ± SD)	22.8 ± 3.9
Resting respiratory rate (breaths min^{-1})	
First exercise session (mean ± SD)	18 ± 5
Second exercise session (mean ± SD)	18 ± 5
Resting heart rate (beats min^{-1})	
First exercise session (mean ± SD)	73 ± 8
Second exercise session (mean ± SD)	72 ± 9
Systolic/diastolic blood pressure (mmHg)	
First exercise session (mean ± SD)	110/71 ± 12/7
Second exercise session (mean ± SD)	109/72 ± 12/7
Estimated cardiorespiratory fitness (METs)	
Maximal METs (mean ± SD)	11.5 ± 2.2

blood pressure were obtained after 5 min of rest. Then, each participant's maximal heart rate (HRmax) was estimated using the age-predicted maximum heart rate in the HRR equation (208 –0.7 × age) (Tanaka et al., 2001), while the targeted heart rates during the exercise stages at 40, 50, 60, 70, 80, and 85% HRR were calculated using the following equation: [HRR (beats min^{-1}) = exercise stages (%) × (HRmax – HRrest) + HRrest] (Cunha et al., 2011).

Next, the participants were equipped with a wireless headset microphone (H8030; Rapoo, Shenzen) and a safety clip on the treadmill before the exercise. The participants could request to stop the exercise test if they experienced discomfort or felt that they could not pursue the test safely, particularly among participants who experienced any deteriorating signs or symptoms such as decreased oxygen saturation less than 85%, persistent irregular heartbeat, muscle cramps, or dizziness. Meanwhile, the standardized instructions for Borg RPE category scale 6 to 20 and the CTT were reviewed for the participants through their headphones to confirm their understanding of the instructions. The instructions and protocols for Borg RPE category scale 6 to 20 and CTT were adopted from Loose et al. (2012), while for tMTT, the standardized instructions were as follows: *"Breath out fully through your mouth. Then, take a deep breath in as much as you can and say out loud at your usual conversational loudness, the following alphabets: | a|, | b|, | c|, | d|, and so on until | z| except | w| .Try to read as many of these alphabets as they appear before having to take another breath. Do not hold your breath when performing this test."* The Borg 6 to 20 RPE chart and the CTT transcript were affixed to a wall in front of the participants, approximately 90 cm away, while the tMTT during the second exercise session was displayed on a seven-inch tablet affixed on the treadmill front rail.

Each participant started walking on the treadmill at 3–4 km/h and 0% elevation for 3 min as a warm-up phase. Then, the treadmill grades were increased by adjusting the speed within a range of 1–4 km/h depending on the participants' tolerance level, and the gradient was increased in 1% intervals until the steady-state targeted heart rates corresponding to 40, 50, 60, 70, 80, and 85% of the HRR were reached (**Figure 1**). As they reached each stage of the exercise, the participants were asked to rate their perceived exertion based on Borg's 6 to 20 RPE scale, while they maintained speed and gradient throughout 2-min bouts of each exercise stage. At the last minute of each exercise stage, they were asked to perform the CTT or tMTT immediately by cueing them with the instruction, "take a deep breath in now and start counting or uttering." During the tMTT, the participants were required to consecutively say out loud as many of the alphabet sounds as possible in a single deep breath and following a pre-set interval of 1 s between the alphabets display. The alphabet that was timed to be uttered was visually cued with font color changes from black into red (**Figure 2**). They had to control their breath by avoiding any attempt to inhale between alphabet phonation before producing speech expirations to the maximum alphabet phonation they were capable of. They were asked to stop their utterances before taking a second breath. Meanwhile, the participants' respiratory rate, oxygen saturation, heart rate, and rhythm were monitored throughout the exercise tests by using a portable patient monitoring system (IntelliVue MX450; Philips, Germany). The speech utterance signals of the CTT and tMTT that were sampled at 44.1 kHz and recorded during the last minute of each 2-min bout of the exercise stages using a wireless microphone were saved into the Praat voice analysis computer software program. Next, data for the utterance rates

FIGURE 1 | A participant performs an incremental exercise on a treadmill until reaching the targeted heart rates at a predetermined percentage of the heart rate reserve.

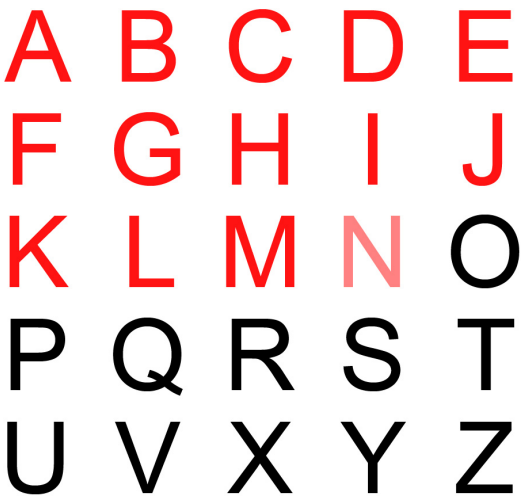

FIGURE 2 | Tablet display of alphabets in the tMTT in front of the participants while they exercised on a treadmill.

were obtained from the Praat script calculation (de Jong and Wempe, 2009) while data for the estimated exercise intensity based on the CTT and the tMTT approaches were measured by determining the percentage of the CTT (%CTT) (Loose et al., 2012) and the percentage of the tMTT (%tMTT), respectively. The %tMTT = ($tMTT_{exercise}/tMTT_{rest}$) × 100, where $tMTT_{exercise}$ is the successful alphabet sounds uttered within a single breath during various stages of exercise while $tMTT_{rest}$ is the successful alphabet sounds uttered at rest before exercise.

Statistical Analyses

The data set was examined for normality by using the Shapiro–Wilk test. Thus, the utterance rate and the estimated exercise intensity for each TT (i.e., %tMTT and %CTT) were compared using a repeated-measure analysis of variance (ANOVA) for the within-subjects variable to identify any differences in %CTT or %tMTT when participants underwent several stages of incremental cardiorespiratory exercise. Spearman's correlation analyses were also performed between the %tMTT and the %HRR, RPE and between the %CTT and the %HRR, RPE measures to evaluate the associations of the variables. All statistical analyses were performed using SPSS Statistics for Windows, v17.0 (IBM), and a probability of $p < 0.05$ was used to determine statistical significance.

RESULTS

Figure 3 shows the different patterns of utterance speech signals over time between the CTT and the tMTT that were produced within a single breath by one of the participants at baseline (rest) and while exercising at moderate (40%HRR) and vigorous stages (60%HRR). Incremental cardiorespiratory exercise had a significant effect on the utterance rates in both CTT [$F(3.2,72.5) = 5.796$, $p = 0.001$] and tMTT [$F(3.3,75.9) = 2.996$, $p = 0.001$]. In both TTs, the utterance rate, which is determined by dividing the total number of syllables by the utterance duration including pauses (de Jong and Wempe, 2009), in all stages of the exercise demonstrated significant differences ($p < 0.05$) in comparison with the utterance rate at baseline (i.e., resting stage) (see **Figure 4**).

The mean CTT at rest (22 ± 4) indicates that the successful phrases participants can utter in a single breath sequentially at rest are until "twenty-two one thousand." Meanwhile, the mean tMTT at rest was 32 ± 8, which denotes that the mean successful monosyllabic alphabets that participants could utter in a single breath sequentially at rest were until the letter/G/in the second round. Although the values at rest in both TTs were incomparable due to the differences in the syllables, the mean duration of utterance at rest for tMTT (31.8 ± 8.3 s) was significantly higher than that for the CTT (20.9 ± 4.4 s), which may indicate that the tMTT encourages participants to achieve further utterances within a single breath while at rest. **Figure 5** shows that both the %tMTT and %CTT decrease as the exercise progresses from lower intensity to higher intensity, which may indicate that the greater anaerobic demand at higher exercise intensity caused more speech difficulty and thus decreased the achieved %tMTT and %CTT. Significant reductions in %CTT were observable at specific transitions of exercise stages, i.e., 36.9% reduction at 40% HRR ($p < 0.001$), 10.3% reduction at 50% HRR ($p < 0.01$), and 6.8% reduction at 70% HRR ($p < 0.01$) in comparison with the preceding stages of exercise. However, no significant changes in %CTT were found between 50 and 60% HRR, between 70 and 80% HRR, and between 80 and 85% HRR. These results have not been reported in previous studies on CTT. In contrast,%tMTT showed significant reductions in transitions to all stages of exercise, with a 47.5% reduction at 40% HRR ($p < 0.001$), 6.9% reduction at 50% HRR ($p < 0.05$), 8.4% reduction at 60% HRR ($p < 0.05$), 5.7% reduction at 70% HRR ($p < 0.01$), 4.5% reduction at 80% HRR ($p < 0.01$) and 4.1% reduction at 85% HRR ($p < 0.001$). Thus, the %tMTT reduction may signify whether participants were at the lower or upper-moderate level of exercise intensity or the lower, intermediate, or upper level of vigorous intensity.

Spearman's correlation analyses were performed between the %tMTT and the %HRR and RPE and between the %CTT and the %HRR and RPE. **Figure 6** illustrates the plots for these correlations, with a correlation coefficient (r_s) of −0.53 for %tMTT and %HRR and −0.51 for %tMTT and RPE ($p < 0.001$ for both correlations). On the other hand, the correlation coefficient between %CTT and %HRR was −0.56 and that between %CTT and RPE was −0.45 ($p < 0.001$) for both correlations) (see **Table 2**). A previous study reported that the correlation coefficient was −0.93 for %CTT and %HRR and −0.86 for %CTT and RPE (Norman et al., 2008).

DISCUSSION

Our results show that only certain cardiorespiratory exercise stages could be distinguished in estimations by the CTT method, but the estimation of exercise intensity by the tMTT method could significantly distinguish all exercise stages. Since CTT may

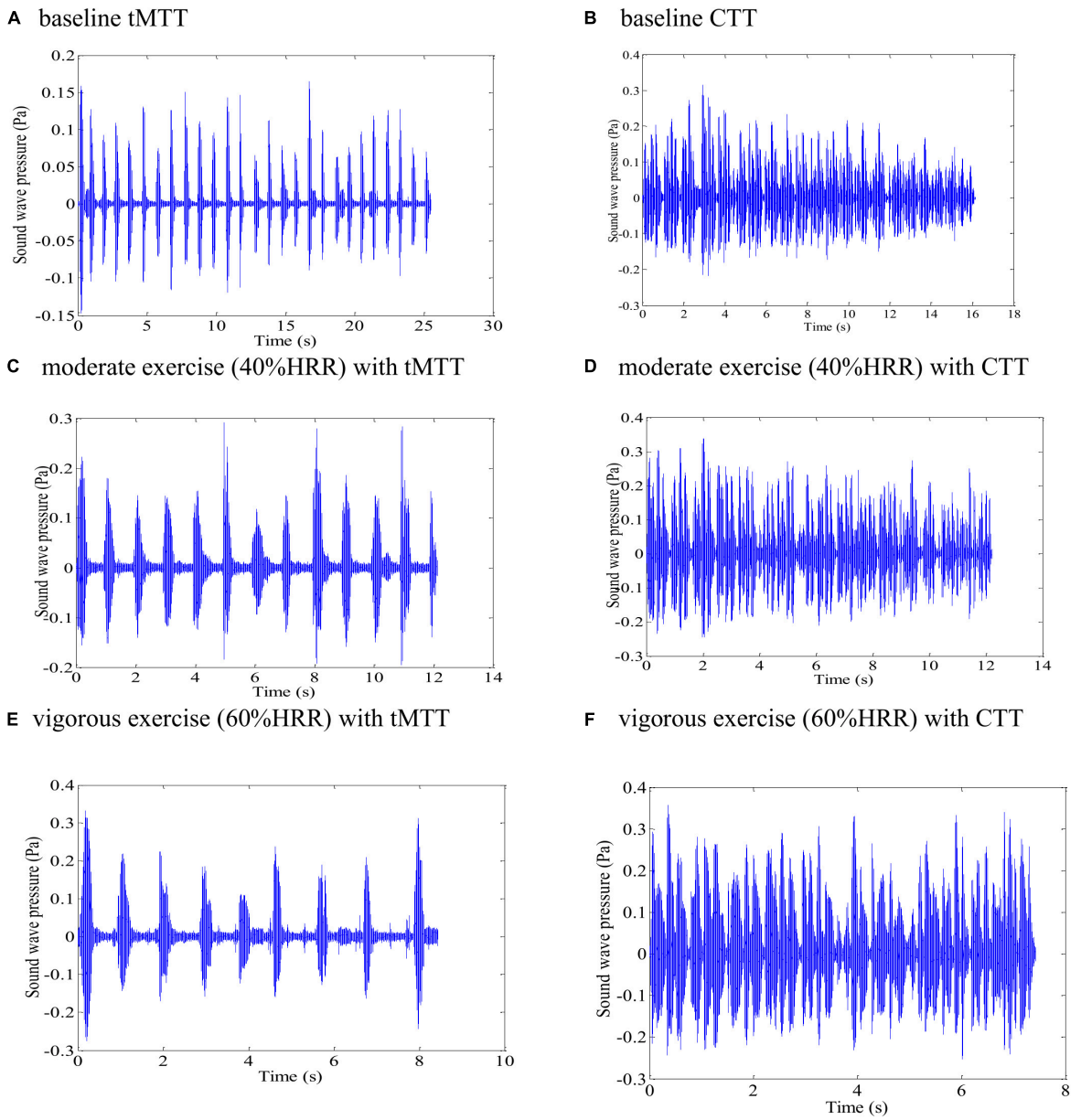

FIGURE 3 | Different patterns of speech signals from one of the participants when producing tMTT versus CTT utterances over time within a single breath at various exercise stages: **(A)** baseline tMTT, **(B)** baseline CTT, **(C)** moderate exercise (40%HRR) with tMTT, **(D)** moderate exercise (40%HRR) with CTT, **(E)** vigorous exercise intensity (60% HRR) with tMTT, and **(F)** vigorous exercise intensity (60% HRR) with CTT.

be preferred by therapists to estimate exercise intensity, this study highlights the importance of standardizing the TT protocol throughout the exercise test to ensure appropriate analysis and interpretation of the exercise intensity achieved by individuals since they considerably function as performance based-outcomes of exercise intensity using a relative measure against the baseline.

Utterance Rate

In tMTT, all 25 letters were initially displayed in black color within a single frame inside the tablet. Each letter that needs to be uttered by the exercising individual changed its color from black to red, one after another at pre-set intervals of 1s (**Figure 2**). The display style and pre-set intervals were chosen due to their high repeatability for the utterance rate and the number of utterances at rest when tested in our preliminary study. Initially, we hypothesize that the utterance rates in the tMTT would be consistent from resting state (baseline) and across exercise stages as it is externally time-controlled while the utterance rates in the self-paced CTT would vary across exercise stages. However, we found that the utterance rates in the tMTT at any exercise stage were significantly different from the baseline utterance rate, but were more likely to be

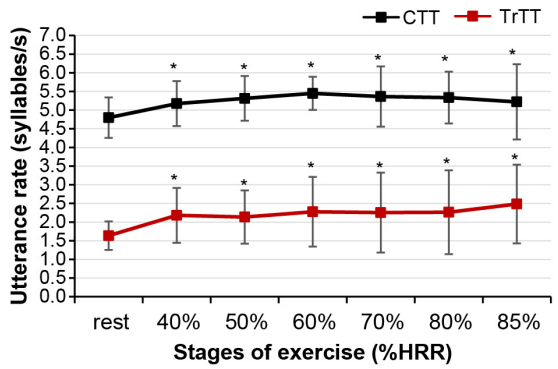

FIGURE 4 | Mean utterance rates in the CTT and tMTT as represented by markers and standard deviation represented by error bars at the resting stage and incremental stages of exercise. Asterisks (*) denote significant differences in the utterance rate between the resting state and the respective stages of incremental exercise at a 0.05 significance level.

consistent across exercise stages. A similar pattern of findings was demonstrated during incremental exercises with the CTT. There are no significant differences in utterance rates in CTT across exercise stages but there was a significant difference between the baseline CTT and the first stage of exercise with CTT (**Figure 4**). It showed that maintaining utterance rates while exercising like in the baseline when there is no exertion, is a challenge regardless of using either the self-pace CTT or the externally controlled tMTT. The utterance rates in tMTT and CTT increase vastly from baseline toward the initial stage of the exercise, which may be attributable to the abrupt increase in ventilation during the initial stage of exercise (Burton et al., 2004; DiMenna and Jones, 2009) and the increased ventilatory oxygen demand for both speaking and exercise tasks (Dempsey et al., 2006; Baker et al., 2008). However, the rates are then maintained throughout exercise stages, probably due to the linear increase in ventilation with the work rate. Moreover, Bailey and Hoit (2002) reported that when both tasks are simultaneously performed, the challenge of coordinating speaking and breathing, especially in a high respiratory drive condition such as during progressive exercise, may interfere with the utterances, but such challenges may not be reflected in the present study as the utterance rates were more likely consistent at certain values for both TTs across stages of incremental exercise.

The timed-control approach in tMTT was initially hypothesized to enable the standardization of TT performance while exercising rather than allowing individuals to utter at their own's pace and because timing is critical to individuals who engage in behavior to anticipate sensory events and prepare appropriate actions (Eagleman et al., 2005). However, the mean utterance rate of tMTT at a vigorous exercise intensity of 85% HRR was found to be as high as 2.5 syllables/s while in other stages of exercise, the utterance rates were maintained approximately at 2.0 syllables/s though the difference was not significant. On contrary, the mean utterance rates of CTT were consistent at approximately 5 syllables/s throughout the exercise stages. The higher utterance rate of tMTT observed at 85% HRR is more likely a response to speaking-related breathlessness and hypercapnic ventilatory drive to breath at higher exercise intensity (Hoit et al., 2007) that cause these participants to speak faster than they supposed to anticipate the time-controlled alphabets utterance in tMTT. Additionally, the anxiety of breathlessness at maximal exercise (Faull et al., 2016) could be a factor of the higher utterance rate of tMTT at the exercise stage of 85% HRR that drives the participants to cease talking while breath-holding and the urge to take another breath.

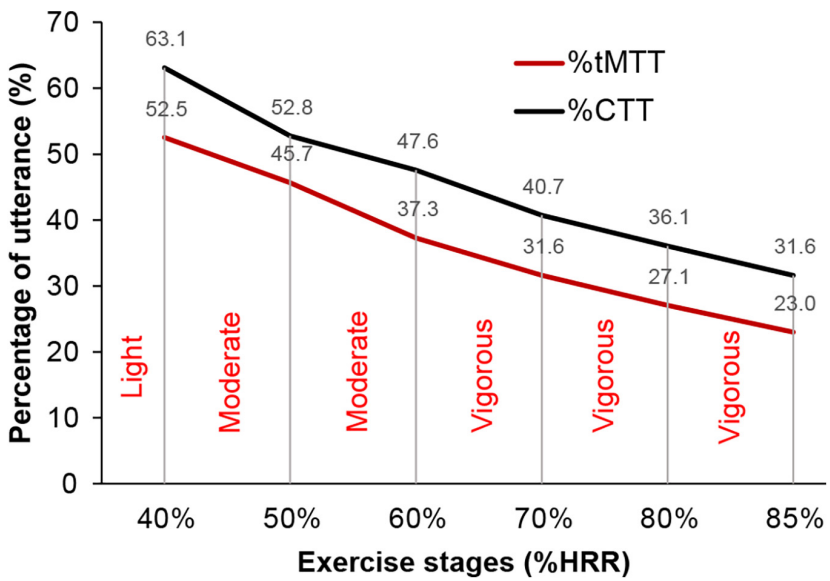

FIGURE 5 | The negative gradient of relative scores (i.e.,%tMTT and %CTT) and exercise stages (%HRR). Intensities of exercise are categorized from light, moderate, and vigorous.

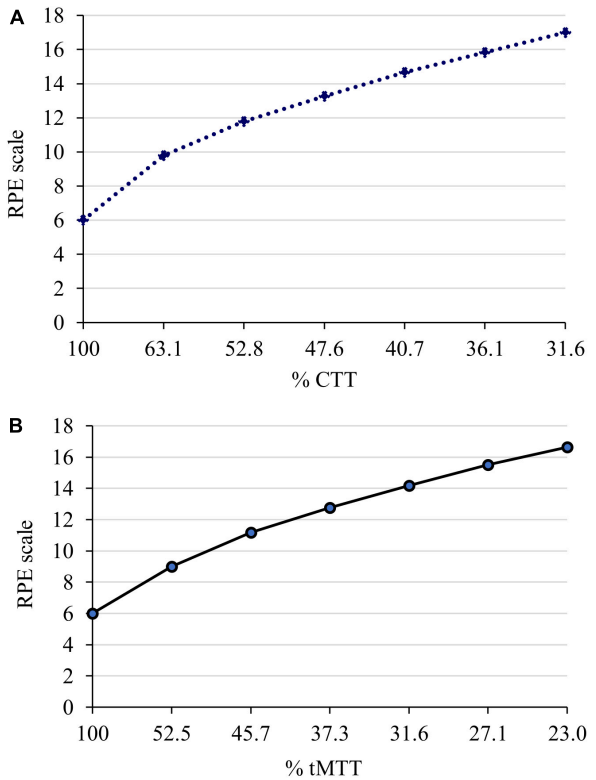

FIGURE 6 | Serial response of perceived exertion based on Borg's 6 to 20 RPE scale during two sessions of incremental cardiorespiratory exercise with **(A)** the CTT and **(B)** the tMTT, respectively. The estimated exercise intensity (%CTT and %tMTT) decreased as the exercise progressed. 100%CTT and 100%tMTT indicate the baseline where RPE scale is the lowest, 6 as there is no exertion.

Fortunately, the anxiety of breathlessness in those nearing maximal ventilation during intense exercise was not prominent in sedentary individuals (Faull et al., 2016) where participants in the present study were among the sedentary population.

Exercise Intensity Estimation

An important finding of the present study is that the %tMTT significantly reduced from one stage of exercise to another in a gradual pattern. This is explained by the fact that ventilation increases linearly with work rates, but when speech and exercise are performed simultaneously, ventilation for speech production is reduced and speech becomes difficult (Meckel et al., 2002; Baker et al., 2008). This is because speech production is limited during the expiratory phases (Meckel et al., 2002; Creemers et al., 2017) and in the competitive ventilatory requirements created by simultaneous phonation and metabolic needs during exercise, non-phonated expirations predominantly occur to remove excess carbon dioxide for important metabolite functions (Bailey and Hoit, 2002), making the speaking task difficult and thus, reducing the utterance output produced.

On the other hand, the %CTT showed no significant changes between exercise stages of 50 and 60% HRR, between 70 and 80% HRR, and between 80 and 85% HRR. These results were not discussed in previous studies related to the CTT, where incremental exercise was set based on HRR progression (Norman et al., 2008; Loose et al., 2012), except in an earlier study by Norman et al. (2002) in which the %CTT was significantly different across exercise workloads corresponding to 50, 60, 75, and 85% of the HRR, which contradicted the findings observed in the present study. These contrasting results could be attributed to the differences in the formulas used for determining the age-predicted maximum heart rate in the HRR equation, wherein the present study equated age-predicted maximum heart rate to (208 − 0.7 × age) (Tanaka et al., 2001) while the previous study by Norman et al. (2002) equated it to a traditional formula (220 – age) before applying it into the HRR equation. The traditional equation for the age-predicted maximum heart rate was previously found to overestimate the maximal heart rates in young adults and increasingly underestimated the maximal heart rates as the participants' age increased, before a revised equation of age-predicted maximum heart rate was introduced by Tanaka et al. (2001) and thus, adopted in the present study. Moreover, our findings suggested that the ability of the CTT to discriminate exercise intensities that are set based on targeted heat rates would probably be influenced by the approach used to determine the maximal heart rate such as a direct measure of maximal heart rate, the traditional equation of age-predicted maximal heart rate, or the revised equation of age-predicted maximal heart rate. Thus, the present study suggests that the tMTT approach could be more appropriately used to quantitatively distinguish the stage of incremental exercise.

Consistent with the study hypothesis, the %tMTT is significantly associated with HRR. The present study demonstrated that exercising at 23 to 53% tMTT would place the participants at a moderate (i.e., 40 to 59% HRR) to vigorous (i.e., 60 to 89% HRR) exercise intensity as described in ACSM's Guidelines for Exercise Testing and Prescription (Pescatello et al., 2014). Moreover, 23% tMTT corresponded to 85% HRR while 53% tMTT corresponded to 40% HRR. Since this is the first study to examine the %tMTT during exercise, the findings obtained with the CTT were used as benchmarks. For a similar group of participants who performed the tMTT and CTT in two separate sessions, 41 to 63% CTT in the present study would correspond to a moderate to vigorous exercise intensity range, where 31% CTT corresponds to 85% HRR and 63% CTT corresponds to 40% HRR. This range of %CTT is higher than the range reported in a previous study (i.e., 33 to 50% CTT) (Norman et al., 2008) that used a similar exercise protocol in young adults. The young adults participating in that study were characterized by cardiorespiratory fitness at maximal

TABLE 2 | Correlation coefficients (r_s) between estimated exercise intensity based on respective percentages of tMTT and CTT against the %HRR and RPE.

Score	Exercise intensity measures		*p*-Values
	%HRR	RPE	
%tMTT	$r_s = -0.53$	$r_s = -0.51$	<0.001
%CTT	$r_s = -0.56$	$r_s = -0.45$	<0.001

(d = 0.51).

oxygen uptake of 46.7 ± 8.1 mL/kg/min (Norman et al., 2008), which is approximately equivalent to 13.3 ± 2.3 METs, while the participants' fitness in the present study was estimated to be 11.5 ± 2.2 METs (**Table 1**). The higher cardiorespiratory fitness seen in the previous study's participants may not justify the lower range of %CTT values when compared to the present study because individuals with excellent aerobic fitness do not usually show dyspnea during exercise without any evidence of pathology. Instead, they are usually well accustomed to the ventilatory demands of exercise Smoliga et al. (2016), and thus may show greater utterance output with a higher %CTT than individuals with poor fitness. However, the cardiorespiratory fitness in the participants of the present study was limited due to estimation from a non-exercise test model with cross validity between 0.72 and 0.8 (Jurca et al., 2005). Meanwhile, the dissimilarity of the %CTT range values in the present study can be postulated to be attributed to the fact that participants in the present study were allowed to see a treadmill control panel that displays workload parameters such as distance, inclination, speed, heart rate, time, and calorie consumption, which were shielded from the participants' view in the previous study (Norman et al., 2008). In the present study, the control panel was kept visible as usual for participants to view it if they wished to, but this was not essential because alterations in the visibility of conscious distance monitoring did not affect the exercise performance (Pinheiro et al., 2011). However, to our knowledge, no previous studies have related the effects of conscious spatial or physiological monitoring on speech output while exercising. This may indicate that shielding the treadmill control panel for spatial and physiological monitoring information, in contrast to the conditions in a usual treadmill exercise, could hinder feedback and induce participants to consciously control their walking or running pace on the treadmill instead of performing the exercise as an automatic action, which could interfere with their exercise performance (Wulf and Prinz, 2001) and thereby affect the corresponding estimation of exercise intensity using %CTT.

Ratings of Perceived Exertion

In line with the study hypothesis, the findings of the present study showed correlations of tMTT with HRR and RPE with medium effect sizes (Field, 2013). Similar effect sizes were observed for correlations between CTT and HRR and between CTT and RPE, while a previous study reported larger effect sizes for similar correlations (Norman et al., 2008). The differences in the effect sizes between the present study and the previous study might be due to the larger sample size in the previous study, which involved 40 participants (Norman et al., 2008). Moreover, the present study showed that when the participants exercised at 50 and 60% HRR (moderate and vigorous exercise intensity), their %CTT was 53 and 48%, respectively, with RPEs of 12 (fairly light) and 13 (somewhat hard), respectively. Likewise, for the same exercise intensity, the %tMTT of the respondents was 46 and 37%, respectively, with RPEs of 11 (fairly light) and 13, respectively and this range of RPE was a recommended exercise intensity for that less trained individuals (Scherr et al., 2013). However, the RPE should be cautiously evaluated as it could increase significantly if the individuals encounter breathing resistance such as face mask-wearing while exercising (Poon et al., 2021) that leads to their discomfort and ultimately affects their perceived exertion. Fortunately, participants in our study did not wear a mask while exercising and performing the TT in a ventilated room.

Practical Implications and Study Limitations

This study highlights an important protocol consideration that, until now, has not been addressed in the literature. First, clinicians need to understand the effects of different TTs on the speech utterance output when these tests are used to estimate and prescribe exercise intensity. Clinicians should also be cognizant when implementing cardiorespiratory exercises to achieve the desired rehabilitation outcomes. For example, if clinicians aim to utilize the self-paced CTT, patients should be able to maintain the required fluency or utterance rate to allow counting of phrases in each bout of exercise and to obtain an accurate utterance output as an estimate of exercise intensity. Our data showed that different stages of incremental exercise will result in significant step-wise reductions in %tMTT, but these reductions are unlikely to appear in %CTT, which will show no significant changes in certain %HRR stages; thus, the exercise intensity may not be well-discriminated when gauged using the self-paced CTT. As a result, the intensity of incremental exercise estimated from %CTT may not correspond to the differences in HR and RPE values as a result of the individual's physiological responses and self-perceived exertion. Additionally, our study only utilized a targeted heart rate based on the individual HRR for each exercise stage. Therefore, the type, length, and rate of utterances used in the TT while performing the incremental cardiorespiratory exercise could result in different physiological outcomes. Thus, clinicians should strive to regulate the utterance rate of the TT across exercise stages to accurately monitor and later prescribe cardiorespiratory exercise. Additionally, it is important to note that step-wise reductions in utterance output are likely to not be perceived at the same rate as step-wise increases in exercise intensity. Since both of these rationales were solely hypothetical and since this study is, to our knowledge, the first study to measure utterance rates during the tMTT and the CTT, future research should further investigate the use of tMTT during cardiorespiratory exercise monitoring. Furthermore, the tMTT correlation was done only on a relatively small sample size of 30 young adults initially, of which only 24 completed the two exercise sessions. However, considering the number of participants (n = 24) and the moderate effect size (d = 0.51) for bivariate correlation in the present study, the statistical power from the *post hoc* power analysis remains acceptable, power (1-β) = 0.84. Thus, the present study still had an 84% chance of yielding a p-value less than 5% (Faul et al., 2007). This statistical power of 84% also exceeds the generally considered minimum desirable value (i.e., 80%) (Araujo and Frøyland, 2007). Lastly, this study did not examine the effects of deep breathing before the TT on the utterance rate and estimated exercise intensity,

and future studies should investigate if these differences in the depth of breaths before the TT translate into improved cardiorespiratory exercise performance. Although this study did not implement a longitudinal design, it is important to translate these acute findings into practice to build on the current body of exercise monitoring and prescription literature.

CONCLUSION

Counting talk test is likely to be sufficient if the determination of different exercise intensity is not a priority, while if the purpose of exercise monitoring is to classify the individuals' exercise intensity as either light, moderate, or vigorous, the tMTT could be more universally employed. However, due to its larger variability in utterance rates across exercise intensities, the tMTT approach may alter the speech breathing of exercising individuals. The conclusion is that future studies are needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Research and Ethics Committee, Ministry of Health Malaysia. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SM participated in all aspects of the present study, data collection, data analysis, and creating the manuscript. RA participated in the data collection. LN and ES participated in the data analysis and manuscript preparation. All authors contributed to the article and approved the submitted version.

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An Alternative Prediction Equation for Evaluation of Six-Minute Walk Distance in Stable Coronary Artery Disease Patients

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Background: As cardio-vascular diseases are the leading cause of death worldwide, establishing measures to improve cardiovascular health is of crucial importance. Exercise plays an essential role in cardiac rehabilitation of patients with coronary artery disease (CAD), in whom an evaluation of the cardiorespiratory fitness (CRF) is necessary. CRF of CAD patients could be assessed using 6-min walk test (6MWT), and the results interpreted by using Enright-Sherill prediction equation which has mainly been designed and evaluated for a healthy population. Hypothesizing that the Enright-Sherill prediction equation might not be best suited for CAD patients, our aim was to reevaluate this equation in CAD patients, and potentially establish a more accurate 6MWD prediction equation to be applied in these patients.

Methods: 6MWD was measured in a cross-sectional study in 67 CAD patients (44 women) who were members of the Coronary club Ljubljana, Slovenia. In addition, the predicted 6MWD was calculated for men and women using Enright-Sherill gender specific regression equation. Multivariate regression analysis was used to obtain a new prediction equation, and the agreement between the measured and the predicted 6MWD analyzed using the repeated measures ANOVA.

Results: Men achieved 451 ± 122 m and women 485 ± 69 m without significant differences between sexes ($F = 0.022$, $p = 0.882$) when adjusted for age, height, body mass, and waist circumference. When comparing the measured (473 ± 91 m) and the predicted (422 ± 57 m) values of 6MWD in CAD patients we found that the Enright-Sherill prediction equation significantly ($F = 27.734$, $p < 0.001$) underestimated the 6MWD by 52 ± 81 m. A significant regression equation was established [$F(3,63) = 44.663$, $p < 0.001$], with a R^2 of 0.680 where 6MWD equals 1,057 m—4.966 x age (years)—0.614 x WC (cm)–68.629 x NYHA class.

Conclusion: The results of this study stress the importance of regular and actual walking ability testing in patients with stable CAD to obtain their CRF, rather than simply predicting it from regression equations obtained from non-representative or non-comparable samples. Our developed prediction equation warrants additional validation and may represent a good substitute for currently used predictions obtained from a healthy population.

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INTRODUCTION

Six-min walk test (6MWT) is a simple field based functional test used to evaluate walking ability in healthy individuals (Casanova et al., 2011) and in patients with various diseases and of different age (Bohannon and Crouch, 2017). The main outcome of the test conducted indoor on a 30-m-long corridor with two turning points is 6-min walk distance (6MWT) (AmericanThoracicSociety, 2002). 6MWT is commonly used in outpatient cardiac rehabilitation programs as a follow-up tool to assess cardio-respiratory fitness (CRF) in patients with coronary artery disease (CAD) and congestive heart failure (Bellet et al., 2012). Studies have reported a good correlation between the 6MWD and clinical status of patients following cardiac rehabilitation, with a minimal clinically important difference of 25 m (Gremeaux et al., 2011), and clinically acceptable reproducibility (Gayda et al., 2004) in CAD patients. It has been suggested that 6MWD can predict future cardiovascular events in patients with stable CAD (Beatty et al., 2012). Moreover, the calculation of gait speed from 6MWT was suggested as a simple risk stratification tool in older CAD patients (Kamiya et al., 2017).

Life-long outpatient cardiac rehabilitation of CAD patients in Slovenia has a 35-year-long tradition of patient organizations (i.e., coronary clubs), which provide supervised exercise training, psychosocial support, access to verified health-related information and continual control of risk factors. The country-wide network encompasses 17 regional coronary clubs located in 81 towns throughout the country, including 153 exercise groups with 3.818 active members (Hadzic et al., 2017). Within those clubs, the 6MWT is performed annually to evaluate CRF of CAD patients. The real achieved 6MWD of a patient is usually compared with the estimated 6MWD, that is calculated from a prediction equation, and the data are presented as a percentage of the predicted value. This comparison is necessary to interpret the fitness status of an individual patient and to modify his exercise plan accordingly.

A thorough systematic review has identified 17 different prediction equations from a healthy population in which the estimated independent predictors of the 6MWD were (depending on the particular equation used) body height, weight, age, sex, and the heart rate assessed during the test, but it was concluded that there are large differences in the predicted distance among the studies (Singh et al., 2014). However, prediction equations based on the predictors obtained from patients are lacking, and the measured values in CAD patients are usually compared to a prediction acquired from a healthy population. Accordingly, evaluation of the CRF based on prediction of 6MWD in an individual patient might be a source of error, as the predicted 6MWD may overestimate or underestimate the true walking ability of the patient, depending on specific equations used in practice. Therefore, we believe that it is necessary to establish a more accurate and specific equation for specific target groups which could be more reliable to use in clinical practice than the commonly used equations based on the data from a healthy population. This is clinically important, as in clinical practice overestimation of patient's ability may lead to adverse cardiovascular events with exercise (Goodman et al., 2013). On the other hand, underestimation may also affect exercise prescription in patients, causing them to exercise with intensity and volume below the threshold necessary to achieve any improvements.

The main goal of our study was to reevaluate the accuracy of the Enright-Sherill equation (Enright and Sherrill, 1998) which is currently used for prediction of 6MWD in CAD patients in regional coronary clubs. Moreover, we aimed to establish a new multivariate regression model including the commonly used independent predictors of 6WMD, potentially applicable for further validation.

In our regression model, we aimed at including some additional predictors that have not been tested before and we believe might improve the accuracy of the predicted 6MWD, such as waist circumference (WC) and the New York Heart Association (NYHA) class of the patient. WC and waist to height ratio (WHtR) were both shown to have the best discriminatory power in predicting cardiovascular risk factors compared to other indices such as body mass, body height and body mass index (Correa et al., 2016). Moreover, NYHA classification (I–IV), based on patient's and physician's assessment of cardiac symptoms including dyspnea, angina, and fatigue at different levels of physical activity, has been mostly used in clinical practice, and studies have stressed a need for a better understanding of the relationship between NYHA class and 6MWD (Yap et al., 2015). We believe that NYHA should be taken into consideration when assessing CRF in CAD patients, and above all, designing their individual rehabilitation program. Accordingly, we hypothesized that NYHA and potentially anthropometric parameters, such as WC and/or WHtR class might importantly contribute to the prediction of 6WMD in CAD patients.

MATERIALS AND METHODS

Participants

This was a cross-sectional study enrolling 67 CAD patients (44 women, 23 men) from the regional coronary club (Ljubljana, Slovenia). All patients have provided written informed consent to participate during their regular annual CRF testing. The study was approved by the Board of Ethics in Sport at the Faculty of Sport in Ljubljana (number 9/2020-491), and accordant to the principles outlined in the Declaration of Helsinki.

Most of the patients had suffered an ischemic heart attack (52%), 24% had percutaneous coronary intervention, and 15% coronary artery bypass grafting. Most common comorbidities were arterial hypertension (65%) and hyperlipidemia (45%).

Patients were classified into different NYHA classes by their treating cardiologist based on clinical appraisal and history of exertion-related symptom onset (I–no limitation of physical activity; II–slight limitation with ordinary physical activity yielding fatigue, palpitation, dyspnea or other cardiovascular symptoms; III–marked limitation; IV–unable to carry out any physical activity without symptoms). There were 11 (16%), 35 (52%) and 21 (31%) patients in NYHA I, NYHA II and NYHA III class, respectively. Patients from NYHA IV class did not participate in the study.

Measurements

All measurements including the 6MWT were performed at the gym of the regional coronary club. Prior to 6MWT, we have measured body height and body mass using a stadiometer and a medical scale (models 222 and 762, respectively; Seca Instruments Ltd., Hamburg, Germany). WC was measured using a tape-meter and according to the WHO STEPwise Approach to Surveillance protocol (STEPS) at the midpoint between the lower border of the rib cage and the iliac crest (Albu et al., 2010).

6MWT was conducted according to the guidelines of the American Thoracic Society (AmericanThoracicSociety, 2002). In general, the test was performed in a 30-m-long corridor free of obstacles, with two turning points and marks placed at a 3-m distance from each other (AmericanThoracicSociety, 2002). Patients were instructed to walk as far as possible for 6 min around the given course, covering as much ground as possible during that time. There was no warm-up prior to the test, and all participants were resting for 10 minutes before starting the test. We have calculated the predicted 6MWD from Enright-Sherill sex specific regressions equations (Enright and Sherrill, 1998) for men and women: for men, 6MWD = (7.57 x height cm)–(5.02 x age)–(1.76 x weight kg)–309 m, and for women, 6MWD = (2.11 x height cm)–(2.29 x weight kg)–(5.78 x age) + 667 m. 6MWD = (0.88 x height cm)–(2.11 x weight kg)–(5.44 x age) + 896 m.

Statistical Analysis

All data were analyzed using the IBM SPSS Software for Windows (version 25, SPSS Inc., Chicago, Illinois, United States). Categorical variables are displayed as numbers and percentages, while continuous variables as means and standard deviations. All numeric variables were firstly checked for normality of distribution with Shapiro-Wilk`s test.

The agreement between the measured and the predicted 6-min walk test distance was first analyzed using Bland–Altman analysis (Ranganathan et al., 2017). Repeated measures ANOVA was used to analyze differences between measured and predicted 6MWD. The differences in 6MWD between different groups (e.g., men vs. women, different NYHA classes, etc) were assessed using univariate analysis of variance adjusted for specified covariates (age, height, body mass and WC). Bonferroni correction for multiple comparisons was used when appropriate. The reported effect size from univariate model was partial eta squared. We have performed repeated linear regression calculation first adding individual predictors and then combining significant ones into the final multiple linear regression model. We have examined potential collinearity, and we present only models with variance inflation factor below 2. A significance level of 0.05 was used for all tests. An *a priori* sample size calculation for multiple linear regression model (fixed model, R^2 deviation from zero) was conducted using G*Power3 (Faul et al., 2009) using a large effect size (d = 0.35), an alpha of 0.05 and three predictors. The result showed that a total sample of 54 participants and critical F value of 2.79 was required to achieve a power of 0.95.

RESULTS

Basic CAD patient characteristics are presented in **Table 1**. The results of the measured 6MWD in CAD patients, classified according to different parameters are presented in **Table 2**.

Men reached 451 ± 122 m and women 485 ± 69 m without significant differences between sexes (F = 0.022, $p = 0.882$) when adjusted for age, height, body mass, and WC. Moreover, even in the unadjusted univariate model there were no statistically significant differences between sexes (F = 2.093, $p = 0.153$). Hence, all further regression analyses were performed without differentiating for sex. On the other hand, the covariates WC (F = 5.07, $p = 0.028$) and age (F = 39.46, $p < 0.001$) were significant predictors of 6MWD in this population.

When analyzing the agreement between the predicted and the measured 6MWD using Bland-Altman plot (**Figure 1**) the prediction equation underestimated the performance in CAD patients for 52 ± 81 m.

This finding was further confirmed when comparing the measured (473 ± 91 m) and the predicted (422 ± 57 m) values of 6MWD in CAD patients using repeated measures ANOVA. We found that the Enright-Sherill prediction equation significantly (F = 27.734, $p < 0.001$) underestimated the 6MWD by 52 ± 81 m. Significantly large effect differences in 6MWD were found for different NYHA classes (F = 14.7, $p <$ 0001, effect size = 0.329) where, as expected, patients from NYHA I were performing significantly better than patients from NYHA II ($p = 0.029$) and NYHA III ($p < 0.001$), and NYHA II patients significantly better than NYHA III patients ($p < 0.001$). We found no significant differences in the measured 6MWD among different BMI ($p = 0.829$) or WHtR ($p = 0.270$) categories.

In the single linear regression, only age and NYHA class were significant predictors of 6MWD (**Table 3**). All multiple regression models including these two predictors were significant, but the highest R^2 was obtained in the model based on age, WC and NYHA class. Based on this model, a significant regression equation was developed [F (3,63) = 44.663, $p < 0.001$], with a R2 of 0.680. Respectively, our predicted 6MWD in patients equals 1,057 m—4.966 x age (years)—0.614 x WC (cm) - 68.629 x NYHA class, where NYHA class is coded as NYHA I = 1, NYHA II = 2, NYHA III = 3, age is measured in years and WC in centimeters (**Figure 2**). 6MWD decreased by about 5 m per each year of age, 0.6 m per every centimeter of WC and NYHA I patients walked approximately 69 and 138 m more than NYHA II and NYHA III patients, respectively. Moreover, the regression model considering only age and NYHA class (but not WC) was also significant, with a R^2 of 0.675.

TABLE 1 | Basic characteristics of the patients with coronary artery disease (CAD).

	Males (*N* = 23)		Females (*N* = 44)		Overall (*N* = 67)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (yrs.)	76.5	7.8	75.1	7.7	75.6	7.7
Body mass (kg)	84.9	12.7	71.2	14.2	75.9	15.1
Body height (cm)	171.5	6.9	158.1	5.1	162.7	8.6
Waist circumference (cm)	104	9	93	11	96.6	11.7
Body mass index (kg/m2)	28.75	3.00	28.34	4.65	28.48	4.14
Waist to height ratio	0.60	0.04	0.59	0.06	0.59	0.06

yrs–years; SD, standard deviation.

TABLE 2 | Six-minute walk distance in coronary artery disease (CAD) patients.

Parameter		**6MWD (meters)**	
		Mean	**SD**
Sex	Men	451	122
	Women	485	69
Test vs. Enright prediction	Measured	473[a]	91
	Predicted	422	57
NYHA classes	Class I	587[b]	31
	Class II	494[c]	28
	Class III	385	100
BMI categories	BMI 18.5–24.9 (normal)	458	96
	BMI 25-0–29.9 (overweight)	471	99
	BMI ≥30 (obese)	487	72
WHtR categories	WHtR <0.5 (no increased risk)	501	20
	WHtR ≥0.5 and <0.6 (increased risk)	497	81
	WHtR ≥0.6 (very high risk)	446	99

NYHA, New York Heart Association; BMI, body mass index; WHtR, Waist to Height Ratio; 6MWD, 6-min walk distance; SD, standard deviation.
[a]*significantly higher measured value; see text for details.*
[b]*significantly higher than NYHA II, and NYHA III; see text for details.*
[c]*significantly higher than NYHA III; see text for details.*

DISCUSSION

The main finding of our study is a new significant regression model explaining 68% of the 6MWD in CAD patients. Accordingly, our prediction equation could be used to evaluate patient performance after conducting a 6MWT. Our results also indicate that: 1) there are no significant differences in 6MWD between men and women with CAD; 2) the Enright-Sherill prediction equation for 6MWD underestimates CAD patient performance on 6MWT; and 3) NYHA class and WC play a significant role in the prediction of 6MWD in CAD patients. To the best of our knowledge, this is the first study to provide such a prediction equation for 6MWD assessment in CAD patients.

Although the Enright- Sherrill prediction equation (Enright and Sherrill, 1998) is adjusted for sex and proposes different calculation of 6MWD for men and women, we found no significant impact of sex on 6MWD in CAD patients. Although studies published in the past 10 years which assessed 6MWT in CAD patients (**Table 4**) were not analyzing sex related differences, it is worth nothing that they did not report 6MWD separately for men and women (Babu et al., 2010; Worringham et al., 2011; Beatty et al., 2012; Gremeaux et al., 2012; Wu et al., 2013; Lv et al., 2015; Yuniadi et al., 2016; Compostella et al., 2017; Waite et al., 2017; Stewart et al., 2018; Rocco et al., 2019; de Bakker et al., 2020). This finding could indicate that a uniform prediction equation should rather be used in the population of CAD without the need to calculate percentage of prediction differently for men and women. Furthermore, a pooled mean 6MWD that we calculated from previous studies (**Table 4**) was 454 ± 85 m and was not significantly different from the one that we are reporting [t (2,666) = 1.777, $p = 0.08$], indicating that the mean 6MWD obtained from our sample is within limits of previously reported values. We would like to highlight that in our study, the mean difference between men and women was 34 m, and that although not significant, this difference was above the reported minimal clinically important difference of 25 m (Gremeaux et al., 2011). However, the study by Gremeaux et al. (2011) included 81 patients of which 77 were men, that could partially explain the discrepancy between the two studies.

Probably the most interesting finding is the fact that the Enright-Sherill prediction equation, obtained from a healthy population, underestimates the measured performance in CAD

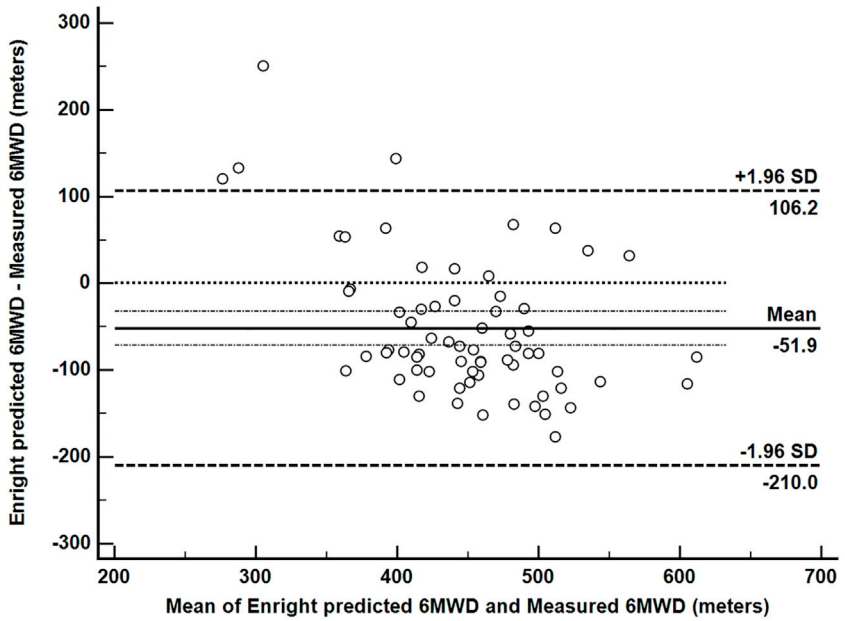

FIGURE 1 | Bland–Altman plot of agreement between the predicted and the measured 6-min walk distance (6MWD). The upper and lower limits of agreement are generally drawn at 1.96 standard deviations (of observed inter-observer differences) above and below the line representing the mean difference (solid line); dotted line is set at zero.

TABLE 3 | Single and multiple linear regression model for the prediction of 6MWD in CAD patients.

Constant distance (meters)		Regression Model		*p*-value	Adjusted R2 (model *p*-value)
	Predictors	Coefficient	95% CI		
1,090	Age	−8.162	929.46; 1,250.95	<0.001	0.477 (<0.001)
442	Body mass	0.421	−1.067; 1.909	0.574	0.005 (0.574)
410	Body height	0.389	−2.234; 3.011	0.768	0.001 (0.768)
608	WC	−1.388	−3.281; 0.505	0.148	0.032 (0.148)
695	NYHA class	−102.593	−125.041; −80.144	<0.001	0.562 (<0.001)
1,181	Age Body mass	−8.583 −0.782	−10.76; −6.397 −1.896; 0.332	<0.001 0.166	0.493 (<0.001)
1,302	Age Height	−8.213 −1.174	−10.59; −6.10 −3.11; 0.76	<0.001 0.230	0.489 (<0.001)
1,218	Age Height WC	−8.213 0.265 −1.734	−10.33; −6.10 −2.08; 2.61 −3.42; −0.05	<0.001 0.822 0.001	0.521 (<0.001)
1,255	Age WC	−8.162 −1.62	−10.31; −6.22 −2.96; −0.28	<0.001 0.019	0.521 (<0.001)
1,057	Age WC NYHA class	−4.966 −0.614 −68.629	−7.020; −2.911 −1.778; 0.549 −93.086; −44.172	<0.001 0.295 0.001	0.680 (<0.001)
989	Age NYHA class	−4.738 −72.607	−6.748; 2.728 −95.889; −49.325	<0.001 0.001	0.675 (<0.001)

6MWD, 6-min walk distance; NYHA, New York Heart Association; WC, waist circumference.

patients by 52 m shown by Bland-Altman limits of agreement analysis, meaning that the two procedures (prediction and actual measurement) cannot be used as substitutes for each other. Considering that the reported minimal clinically important difference for 6MWD is 25 m (Gremeaux et al., 2011), we may conclude that the underestimation is both statistically ($p < 0.001$)

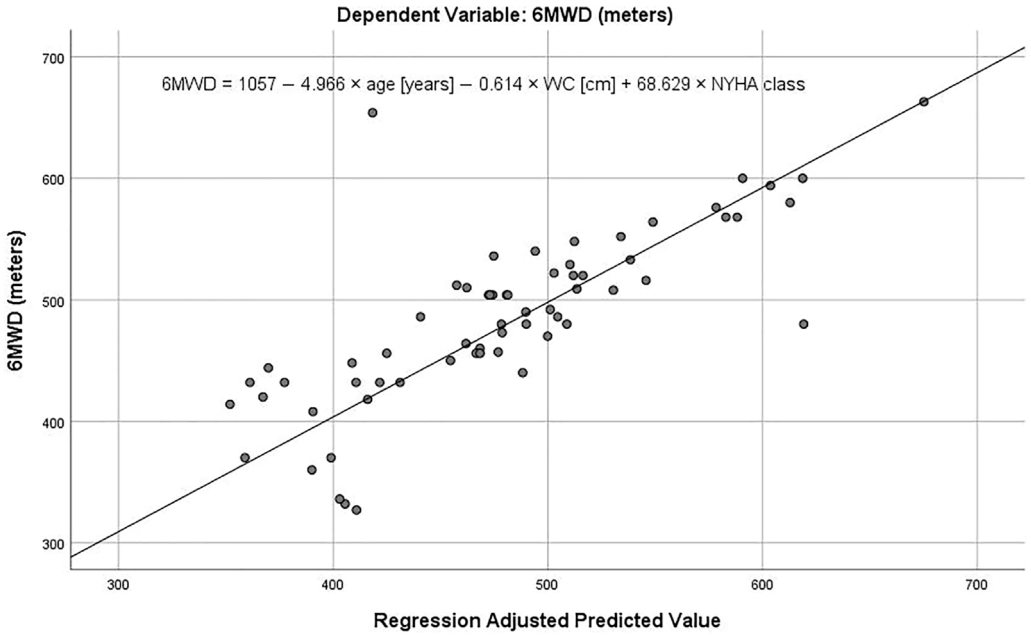

FIGURE 2 | A prediction of the 6-min walk distance (6MWD) in the adjusted multiple linear regression model. WC–waist circumference; NYHA–New York Heart Association. See text for details.

TABLE 4 | Six-minute walk distance (6MWD) in patients with coronary heart disease.

Author	Year	*N*	Sex	6MWD (meters)	
				Mean	Std. dev
Current study	2020	65	males/females	473	91
de Bakker	2020	607	males/females	563	77
Stewart	2018	875	males/females	340	117
Rocco	2019	52	males/females	443	62
Waite	2017	11	males/females	237	147
Compostella	2017	139	males	520	114
Gremeaux	2011	81	males/females	488	NA
Yuniadi	2016	26	males/females	352	90
Lv	2015	43	males/females	513	94
Wu	2013	34	males/females	439	87
Beatty	2013	556	males/females	481	36
Gremeaux	2012	30	males/females	490	33
Worringham	2011	134	males/females	524	NA
Babu	2010	15	males/females	470	151

and clinically significant. When 6MWT is performed in outpatient setting, the results are interpreted to patients as a percentage of the predicted value of 6MWD, which means that in our case we would wrongly classify patient's CRF for no objective reason. This may decrease patient's motivation for exercise, as the patient may wrongly percept his performance as clinically acceptable, although he might indeed perform better. Therefore, we conclude that additional properly powered studies are warranted using this proposed, population specific prediction equation to evaluate if it is a more appropriate approach for evaluation and follow-up of CRF in CAD patients. Moreover, the fact that the predicted 6MWD assessed by Enright-Sherill equation underestimated the real 6MWD in patients puts into question the relevance of the equation also in a healthy population and exposes a need for a re-evaluation.

As expected, NYHA class significantly affected the CAD patients' performance (**Table 2**). In NYHA 1, where there is no limitation of physical activity and where moderate physical activity does not cause undue fatigue, palpitation and/or dyspnea, the 6MWD was 587 ± 31 m. In NYHA II, where slight limitation of physical activity and comfort at rest exist, the 6MWD was below 500 m (494 ± 28 m), and in NYHA III where there are marked limitations of physical activity with important cardio-respiratory symptoms, 6MWD was below 400 m (385 ± 100 m). In a previous study (Yap et al., 2015), authors did not report any significant differences between NYHA I and II (420 vs. 393 m; $p = 0.416$), but they did report significant differences in mean 6MWD between NYHA II and III (393 vs. 321 m; $p = 0.014$) and III and IV (321 vs. 224 m; $p = 0.027$), respectively. In the best regression prediction model obtained in our study the performance in 6MWT has decreased by 69 m for each change in NYHA class. This has not been shown in any study performed so far. Based on this finding we believe that clinical classification of the heart function performed by cardiologist is crucial to predict and evaluate CRF in this population.

Our calculated regression model that includes age, NYHA class and WC has explained 68% of the variance (with a $R^2 = 0.680$) of 6MWD in CAD patients which is much better than the 40% variance explained by the Enright-Sherill equation obtained in a healthy population. Compared to other prediction equations based on a healthy population data (Singh et al., 2014) where R^2 ranged from 0.09 to 0.77, only three studies reported an equation

with R^2 larger than 0.68 (Poh et al., 2006; Ben Saad et al., 2009; Casanova et al., 2011).

Finally, we would like to stress some strengths and limitations of our study. We have included several important predictors that have not been used previously. We believe that the proposed equation is very feasible given that all predictors are assessed routinely. However, although powered enough the sample size in our study was still relatively small ($N = 66$; sample size calculation was $N = 54$). Nevertheless, seven of 13 similar studies conducted so far (**Table 4**) included a smaller sample size. Although some could still consider our sample size as a limitation for the accuracy of estimated regression coefficients, studies have also confirmed that even two events per variable can be enough for adequate estimation of regression coefficients, standard errors, and confidence intervals (Austin and Steyerberg, 2015). Another thought worth noting is the fact that in our sample, the number of female participants far outranged the number of male participants, although the prevalence of cardiovascular diseases is usually higher in males. The sex difference in prevalence of CAD diminishes in older age. In addition, it seems that less males than females usually participate in rehabilitation programs, and females are more adherent. This observation urges for additional studies encompassing larger, population representative samples and stresses the need for a more efficient promotion of importance of cardiovascular rehabilitation, especially targeting male population.

In conclusion, we have provided a new prediction equation based on multivariate regression model for 6 MWD estimation, considering the variables age, NYHA class and WC as predictors. To the best of our knowledge, this is the first study to challenge prediction of 6MWD using this approach. The prediction equation developed in this study may represent a good substitute for currently used predictions from healthy population in order to avoid the possibility of underestimation or overestimation of patient performance. However, it should be stressed that our results are not providing evidence for this, as analysis of proposed equation in an independent CAD patients sample is necessary for validation and potential clinical use. Nevertheless, the results of our study stress the importance of regular and actual walking ability testing in patients with stable CAD to obtain their CRF, rather than simply predicting it from regression equations obtained from non-representative or non-comparable samples. Actual testing and better data interpretation using more objective prediction equation enables a more realistic and obtainable exercise goal setting in CAD patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Board of Ethics in Sport at the Faculty of Sport in Ljubljana (number 9/2020-491). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, HL, AN, and VH; Investigation, AN, DK, and MV; Methodology, VH, HL, MS, and BJ; Formal analysis, VH; Original draft preparation, HL and VH, Writing—review and editing, HL, AN, BJ, ED, DK, MV, MS, and VH. All authors have read and agreed to the published version of the manuscript.

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REVIEW
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Fractal Correlation Properties of Heart Rate Variability as a Biomarker for Intensity Distribution and Training Prescription in Endurance Exercise: An Update

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While established methods for determining physiologic exercise thresholds and intensity distribution such as gas exchange or lactate testing are appropriate for the laboratory setting, they are not easily obtainable for most participants. Data over the past two years has indicated that the short-term scaling exponent alpha1 of Detrended Fluctuation Analysis (DFA a1), a heart rate variability (HRV) index representing the degree of fractal correlation properties of the cardiac beat sequence, shows promise as an alternative for exercise load assessment. Unlike conventional HRV indexes, it possesses a dynamic range throughout all intensity zones and does not require prior calibration with an incremental exercise test. A DFA a1 value of 0.75, reflecting values midway between well correlated fractal patterns and uncorrelated behavior, has been shown to be associated with the aerobic threshold in elite, recreational and cardiac disease populations and termed the heart rate variability threshold (HRVT). Further loss of fractal correlation properties indicative of random beat patterns, signifying an autonomic state of unsustainability (DFA a1 of 0.5), may be associated with that of the anaerobic threshold. There is minimal bias in DFA a1 induced by common artifact correction methods at levels below 3% and negligible change in HRVT even at levels of 6%. DFA a1 has also shown value for exercise load management in situations where standard intensity targets can be skewed such as eccentric cycling. Currently, several web sites and smartphone apps have been developed to track DFA a1 in retrospect or in real-time, making field assessment of physiologic exercise thresholds and internal load assessment practical. Although of value when viewed in isolation, DFA a1 tracking in combination with non-autonomic markers such as power/pace, open intriguing possibilities regarding athlete durability, identification of endurance exercise fatigue and optimization of daily training guidance.

Keywords: heart rate variability, aerobic threshold, DFA a1, exercise intensity distribution, endurance training

INTRODUCTION

In an attempt to determine physiologic thresholds for exercise intensity distribution, various methods have been employed such as gas exchange transitions, blood lactate and heart rate (HR) variability (HRV) kinetics (Meyer et al., 2005; Michael et al., 2017; Jamnick et al., 2020; Poole et al., 2021). The first boundary transition has been described as the aerobic threshold (AeT) represented by the first ventilatory (VT1) or blood lactate (LT1) thresholds (Meyer et al., 2005; Faude, 2009). The second boundary has been referred to as an anaerobic threshold (AnT) represented by the second ventilatory (VT2) or blood lactate (LT2) thresholds (Meyer et al., 2005; Keir et al., 2015). Threshold detection derived from HRV has been extensively investigated over the past 25 years since HR monitoring is a relatively straightforward, non-invasive, low cost option available to the general public (Michael et al., 2017). However, despite early enthusiasm, conventional HRV indexes have not been widely embraced for threshold or intensity assessment by either researchers, athletic monitoring device vendors, coaches or athletes. Part of this reluctance stems from two key issues. Traditional HRV indexes such as time domain parameters like standard deviation of corrected RR intervals (SDNN) or the standard deviation 1 from Poincaré plot analysis (SD1) do change during dynamic exercise but approach a nadir at around the AeT (Gronwald et al., 2020). Therefore, to determine the AeT, a formal incremental exercise test to near exhaustion is needed to demonstrate that nadir. Perhaps the more troublesome issue concerning a comprehensive intensity classification framework based on HRV is one of index dynamic range during endurance exercise. Since a nadir occurs at the AeT, there is no further information to be gained from observation past this region, making them poorly suited for spanning the full intensity range of exercise. Conversely, a dimensionless index of HRV based on fractal correlation properties (short-term scaling exponent alpha1 of Detrended Fluctuation Analysis: DFA a1) has been shown to have a wide dynamic range encompassing the low, moderate and high exercise intensity domains (Gronwald et al., 2020; Gronwald and Hoos 2020). Given these properties, a proposal was made to utilize this index as a biomarker for exercise intensity distribution including discrete numerical values that correspond to physiologic threshold boundaries (Gronwald et al., 2020). Since that hypothesis was made, some key findings have been published both supporting and expanding on this promising concept. This update will summarize progress to date and explore areas that still need further investigation.

WHAT ARE FRACTAL CORRELATION PROPERTIES OF HRV?

Fractals are considered complex structures that possess self-similarity at various degrees of magnification. Natural spatial examples of fractal structures include coastlines, snowflakes or tree branchings. No matter the scale or magnification, the essential underlying pattern is similar (Eke et al., 2002). Fractal behavior of the cardiac beat series is characterized as degrees of self-similarity of the beat sequence over different time scales (Goldberger et al., 2002). DFA a1 is based on these fractal cardiac beat arrangements which can also be embodied as "correlation properties" of the pattern over short time spans. To better understand the concept of correlation properties, analogies to a random walk have been used (Hardstone et al., 2012). For example, during a random walk, at each next step, the walker can choose to go either right or left. If the choice the walker makes is not random but based on the previous sequence (series of right or left decisions), the pattern is described as being well "correlated" (DFA a1 near 1.0), since the future pattern is based on the past history. Values above 1.0 denote progressively higher degrees of correlation. But, if each new step is taken with equal, random chances of right or left, an "uncorrelated" pattern exists (DFA a1 of 0.5). During exercise it has been observed that at low intensity, DFA a1 values are usually in a correlated range near or above 1.0 (Gronwald et al., 2020; Gronwald and Hoos, 2020). As intensity rises, DFA a1 declines, passing 0.75 at moderate loads, continuing to drop further past the 0.5 range with increasing exercise intensity (uncorrelated random behavior of interbeat pattern), finally to drop below 0.5 (representing an anticorrelated range) at the very highest work rates. Anticorrelated behavior refers to a pattern that tends to bring the walker back to midline and can be viewed as an immediate self-correction mechanism associated with the potential failure of homeodynamic regulation and can only be tolerated for short time spans (Karasik et al., 2002). These correlation patterns are felt to be due to changes in sinoatrial pacemaker function under control by the balance between the reciprocal branches of the autonomic nervous system (ANS) (Michael et al., 2017). During exercise there is both a withdrawal of parasympathetic and enhancement of sympathetic activity resulting in a change of HRV including DFA a1 (White and Raven, 2014). Therefore, alterations in DFA a1 provide a view of autonomic balance from rest to severe intensity domains. This autonomic based index of systemic internal load contrasts with established markers of intensity that depend on physiologic subsystems such as cardiorespiratory variables (VO_2, VCO_2), chemical moieties (lactate) and measures of external load (speed/power).

DO SPECIFIC VALUES OF DFA A1 CORRESPOND TO CONVENTIONAL PHYSIOLOGIC EXERCISE THRESHOLDS?

Previous studies exploring DFA a1 behavior through progressive increases in exercise intensity have shown that at workloads near the AeT, index values fall midway between well correlated (1.0) and uncorrelated states (0.5) (Gronwald et al., 2020). Explanations for the association of physiologic breakpoints with correlation properties of HRV can revolve around practical observations (empirically derived from observing DFA a1 *vs.* VT1/LT1 during exercise ramp or stage studies) but can also be understood from a network physiology standpoint as an integrated concept of ANS regulation during endurance exercise (Balagué et al., 2020; Gronwald et al., 2020). Network

physiology encompasses multiple neuromuscular, biochemical, peripheral and central nervous system inputs leading to an overall concept of "organismic demand" that is reflected in correlation properties of HRV and consequently in the response of DFA a1. Therefore, it was conjectured that a specific value of DFA a1 may correspond to the AeT, which would be helpful in sports and exercise science given the importance of this training boundary for intensity distribution models in many fields of application (Gronwald et al., 2020). Whether the desired program is polarized, pyramidal or threshold in type, identification of the low intensity boundary would be necessary (Seiler and Kjerland, 2006; Esteve-Lanao et al., 2007; Stöggl and Sperlich, 2015, 2019; Bourgois et al., 2019). With this objective in mind, the question of whether a value of DFA a1 between correlated and uncorrelated corresponds to the VT1 was evaluated in a group of male recreational runners (Rogers et al., 2021a). Results indicated that reaching a DFA a1 of 0.75 during an incremental treadmill test was associated with the VT1 and termed the heart rate variability threshold (HRVT). While this finding was encouraging, widespread application as an AeT boundary requires support in many demographic groups. Therefore, a very different class of participant, comprising male cardiac disease patients (congestive heart failure, stable coronary disease) was studied using an incremental cycling ramp protocol (Rogers et al., 2021f). During the cycling test the HR and VO_2 attained at the VT1 was strongly associated with the HR and VO_2 at the HRVT. Finally, in a contrasting population, the HR and cycling power at the HRVT was associated with the HR and cycling power derived from the LT1 in a group of elite triathletes (7 male, 2 female) performing an incremental cycling stage protocol (Rogers et al., 2022a). Although female data on DFA a1 behavior is sparse, the 2 female participants in this group had typical DFA a1 responses to incremental cycling exercise. In addition, it was also hypothesized that another physiologic breakpoint, the AnT, could occur at a DFA a1 of 0.5 (Rogers et al., 2021c). This value is associated with the transition from an uncorrelated to an anticorrelated pattern in HR time series. Since the anticorrelated state is felt to be an autonomic response indicating organismic destabilization (Seely and Macklem, 2004), it could correspond to a parallel phenomenon represented by a loss of cardiorespiratory sustainability. In support of this belief, study results from a recreational runner cohort showed that reaching a DFA a1 of 0.5 was associated with that of the VT2 and termed as the second heart rate variability threshold (HRVT2) (Rogers et al., 2021c). Most recently, a study done by Mateo-March et al. (2022) showed good agreement and correlation with both the HRVT and HRVT2 with lactate derived first and second thresholds in a large group of male professional cycling participants. Although the LT2 to HRVT2 correlation was high ($r = 0.93$ for cycling power, $r = 0.71$ for HR) there was a statistical difference in mean values (bias of 8 W or 4 bpm). As in Rogers et al. (2021c), the limits of agreement for the HRVT2 were relatively wide. Further studies should examine this more closely and attempts made to improve individual variations.

Although prior studies indicated that DFA a1 declines as external exercise load rises, none had previously attempted to establish a distinct value corresponding to the AeT or AnT. Part of this difficulty relates to the method of DFA a1 plotting used during incremental testing. In previous work, DFA a1 behavior was routinely assessed by using non-overlapping measurement windows (of 1–5 min) often at the end of each intensity stage or condition of exercise (Gronwald and Hoos, 2020). Therefore, if an incremental exercise test consisted of 9 stages of 30 W per stage (10 w/min rise), only 9 DFA a1 data points would be available. To better detect a more precise pattern in DFA a1 behavior, a different method of DFA a1 plotting was utilized. This technique used fixed measuring windows of 2 min but did a rolling, ongoing recalculation every 5 s of activity (Kubios HRV Premium software "time varying" option: window width of 2 min, grid interval of 5 s). The 2-min time windowing was chosen based on the calculations by Chen et al. (2002) to achieve a sufficient number of RR data points to achieve DFA a1 validity. By using this method, a nearly straight-lined drop of DFA a1 from values of approximately 1.0 to 0.5 became apparent (Rogers et al., 2021a), providing an opportunity for simple linear interpolation of the corresponding HR or time plotted against DFA a1 of 0.75 (see **Figure 1**). Although not extensively studied, it also appears that constant power cycling intervals with 2-min measurement windows may also be used for HRVT determination (Gronwald et al., 2021). Another group looking at DFA a1 behavior in recreational runners using a 5-min measurement window showed similar correspondence to physiologic exercise threshold results during treadmill intervals with DFA a1 values of 0.68 ± 0.28 for the VT1 and 0.48 ± 0.11 for the VT2 (Naranjo-Orellana et al., 2021). Thus, DFA a1 values of 0.75 and 0.5 may represent a comprehensive solution to accepted physiologic exercise boundaries across a wide spectrum of individuals. In terms of individual participant agreement between HRV and gas exchange/blood lactate derived thresholds, they appear to be of similar magnitude to that of other comparisons of threshold approaches such as blood lactate versus ventilatory parameters (Pallarés et al., 2016), assessment of gas exchange techniques for VT1 determination (Gaskill et al., 2001), comparison of the maximal lactate steady state (MLSS) and functional threshold power (FTP) (Klitzke Borszcz et al., 2019) as well as the muscle oxygen desaturation breakpoint association to the MLSS (Bellotti et al., 2013). Despite the validation with established threshold concepts, it should be kept in mind that the present systemic approach is based on ANS regulation that does not necessarily match perfectly with other concepts based on subsystem parameters.

DFA A1 INTERNAL LOAD ASSESSMENT BEYOND PHYSIOLOGIC EXERCISE THRESHOLD TESTING

Although exercise threshold awareness is of great importance, status of ANS regulation over time may provide key information about the level of internal load especially when the HR *vs.* VO_2 or HR *vs.* power relationship is skewed. This is best exemplified by the process of eccentric cycling (Barreto et al., 2021). This activity is based on actively resisting motorized bicycle pedal motion moving in a reverse direction, thereby applying muscular force in

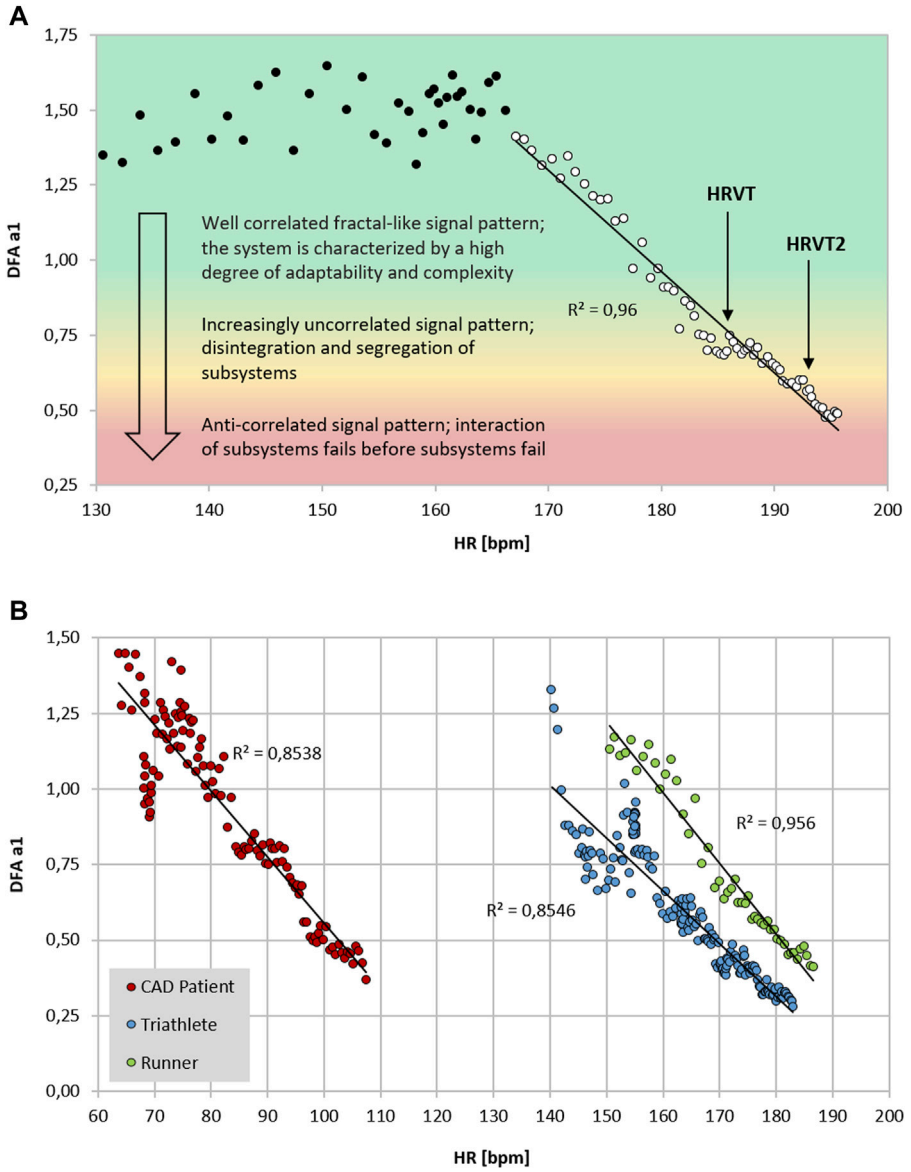

FIGURE 1 | (A) DFA a1 *vs*. HR of a 26-year-old male runner with a VO_{2MAX} of 72 ml/kg/min, HR at VT1 of 183 bpm and HR of 192 bpm at VT2 performing an incremental treadmill ramp test, including a qualitative description of the signal pattern; data recorded with an ECG (MP36; Biopac Systems Ltd., Essen, Germany) (data from Rogers et al., 2021a; Rogers et al., 2021c). Data processed in Kubios HRV Premium software (Version 3.5) using automatic correction method (artifact percentage: < 5%). Shading indicates a 3 zone exercise intensity model defined by DFA a1 thresholds. **(B)** DFA a1 *vs*. HR of three participants during incremental exercise ramps (data from Rogers et al., 2021f; Rogers et al., 2022a; Rogers et al., 2021a respectively). Data processed in Kubios HRV Premium software (Version 3.5) using automatic correction method (artifact percentage: < 5%). Red circle: 59-year-old male with stable coronary artery disease (CAD), beta blocker usage and a VO_{2MAX} of 25 ml/kg/min, HR at VT1 of 83 bpm and HR at VT2 of 109 bpm performing an incremental cycling ramp test; data recorded with an ECG (VISTA Holter NOVACOR, Rueil, Malmaison, France). Blue circle: 23-year-old female triathlete with a VO_{2MAX} of 60 ml/kg/min, HR at LT1 of 154 bpm and HR at LT2 of 165 bpm performing an incremental cycling stage test; data recorded with Polar H10 chest strap (Polar Electro Oy, Kempele, Finland). Green circle: 19-year-old male runner with a VO_{2MAX} of 58 ml/kg/min, HR at VT1 of 167 bpm and HR at VT2 of 179 bpm performing an incremental treadmill ramp test; data recorded with an ECG (MP36; Biopac Systems Ltd., Essen, Germany).

an eccentric fashion. During eccentric cycling at the same VO_2, an individual is usually able to pedal at 3x the power as conventional cycling. Therefore, at equivalent metabolic cost, eccentric cycling can be performed at much higher power levels, resulting in enhancement of muscular size, strength, and oxidative properties (LaStayo et al., 2000). This is particularly advantageous in groups with low cardiovascular fitness who would benefit from improved functional muscle mass such as those with heart failure or pulmonary disease (Gremeaux et al., 2010). Although there appears to be distinct benefits to eccentric training, assessing intensity distribution is problematic. This is due to both the power and HR discrepancy measured from

conventional physiologic exercise thresholds compared to those determined by concentric cycle testing (Barreto et al., 2021). HR is usually higher at equivalent VO_2 in eccentric compared to concentric cycling possibly associated with a reduction in cardiac stroke volume (Ritter et al., 2019). Additionally, many of the ideal candidates for eccentric training have cardiac disease and may be on beta adrenergic blocking medication, further complicating conventional HR targets. An initial exploration of DFA a1 behavior during prolonged low intensity unaccustomed eccentric cycling indicated that some participants had major suppression of DFA a1 into the anticorrelated range associated with substantial autonomic perturbation (Rogers et al., 2021d). Therefore, in exercise types that do not conform to standard exercise intensity models, DFA a1 could provide a measure of internal load and safety in "at risk" populations.

CAN DFA A1 BE USED AS AN INDEX OF FATIGUE, DAILY TRAINING GUIDANCE OR DURABILITY?

DFA a1 as an Index of Endurance Exercise Fatigue and Daily Directed Training

Though recognized measures of endurance exercise induced fatigue have been explored, none are of practical value while in the midst of an exercise session or race. There are also questions regarding the validity of heart rate drift as a sign of fatigue during exercise (Maunder et al., 2021). HR drift appears to be a complex phenomenon (Souissi et al., 2021) that can normalize or even reverse with very long endurance efforts (Mattsson et al., 2011). Established performance indices or biomarkers such as counter movement jump (CMJ) height, running economy, muscle enzyme elevation like creatine phosphokinase (CPK), salivary hormones, markers of substrate availability, blood lactate concentration and cortical activity (Jastrzębski et al., 2015; Knechtle and Nikolaidis, 2018; Martínez-Navarro et al., 2019; Wu et al., 2019) are mainly used after the activity, during interval sessions or as monitoring tools at periodic times during standardized rest conditions. While regular HRV monitoring at rest has been proposed as a means to prevent functional overreaching and assess baseline autonomic balance (Stanley et al., 2013), no HRV index has been shown to have the ability to demonstrate fatigue while performing exercise. Since DFA a1 possesses dynamic range through all exercise intensity zones, a divergence between an indicator of autonomic status (e.g., DFA a1) and measures of external load (e.g., power/pace) could potentially be used as an indication of fatigue while still performing the activity. For instance, if the DFA a1 is usually 0.75 at a pace representing the AeT in a well-rested individual, would it be different after lengthy endurance exercise? To help answer this question an examination of running economy, HR, CMJ and DFA a1 in a group of experienced ultramarathon participants was explored before and after a 6-h trail based run (Rogers et al., 2021e). Seven athletes performed a 5-min treadmill test (at or below VT1 intensity) before and after the 6-h session. Results showed a significant DFA a1 decline from baseline after running for 6 h but without major change in HR or running economy. Additionally, mean DFA a1 in the post run group was 0.32, signifying an anticorrelated value seen with the highest intensity exercise domains despite a pace below the AeT. The fatigued state was confirmed by showing a significant impairment in CMJ height after the 6-h run. Interestingly, no HR change was noted in this study, similar to findings of Mattsson et al. (2011). Therefore, observation of inappropriately suppressed DFA a1 at an exercise intensity previously shown to be associated with well correlated values, can be potentially viewed as an autonomic indicator of fatigue.

In a monitoring purpose the usage of DFA a1 may help inform an athlete about their recovery from previous training sessions. It has become commonplace for individuals to monitor and track resting HRV as a method to direct daily exercise intensity and volume (Granero-Gallegos et al., 2020; Düking et al., 2021). Unfortunately, resting HRV requires a regular day-to-day monitoring routine including standardization (e.g., time of day, nutrition; Bellenger et al., 2016) and logistically may not fit into an irregular schedule. As resting HRV is a reflection of autonomic balance post exercise (Seiler et al., 2007), the observation of DFA a1 early in the upcoming exercise session could also provide similar clues. In other words, DFA a1 relative to power/pace could provide insight into current systemic recovery status while still in a low intensity warm-up stage. If relative suppression of DFA a1 is seen at a low exercise intensity previously associated with well correlated fractal patterns, it can be interpreted as signifying inappropriate autonomic balance. With this in mind, it is also important to realize that DFA a1 based physiologic exercise threshold testing performed in a fatigued, overreached or ill individual may not be similar to testing when healthy and well rested.

Durability Assessment Using DFA a1 With Measures of External Exercise Load

Extrapolation of the observation above could also lead to usage of DFA a1 as a marker of both "exercise durability" and as a method for daily decisions about "training readiness". In a recent publication, athlete "durability" was described as "the time of onset and magnitude of deterioration in physiological-profiling characteristics over time during prolonged exercise" (Maunder et al., 2021). In other words, durability is an assessment of fatigue related reduction in performance, as opposed to standard measures of athletic fitness such as VO_{2MAX} or maximal lactate steady state. To assess durability, quantifying the starting point and degree of performance decline due to fatigue is needed. Leveraging DFA a1 as an index of overall "organismic demand" in conjunction with simultaneous measures of external exercise load could help assess this performance decline from an ANS perspective. In this context, DFA a1 would reflect changes/deterioration in autonomic balance seen at a particular pace/power during exercise. Since DFA a1 values span the spectrum of exercise intensity, the assessment of autonomic imbalance can be made whether the internal load condition is high or low. An example of this is shown in **Figure 2A** where DFA a1 is markedly suppressed at a pace well below the VT1 after the aforementioned 6-h run without changes in HR. In a second example (**Figure 2B**), there was no major change in DFA a1 behavior during incremental cycling exercise before and after a 2-

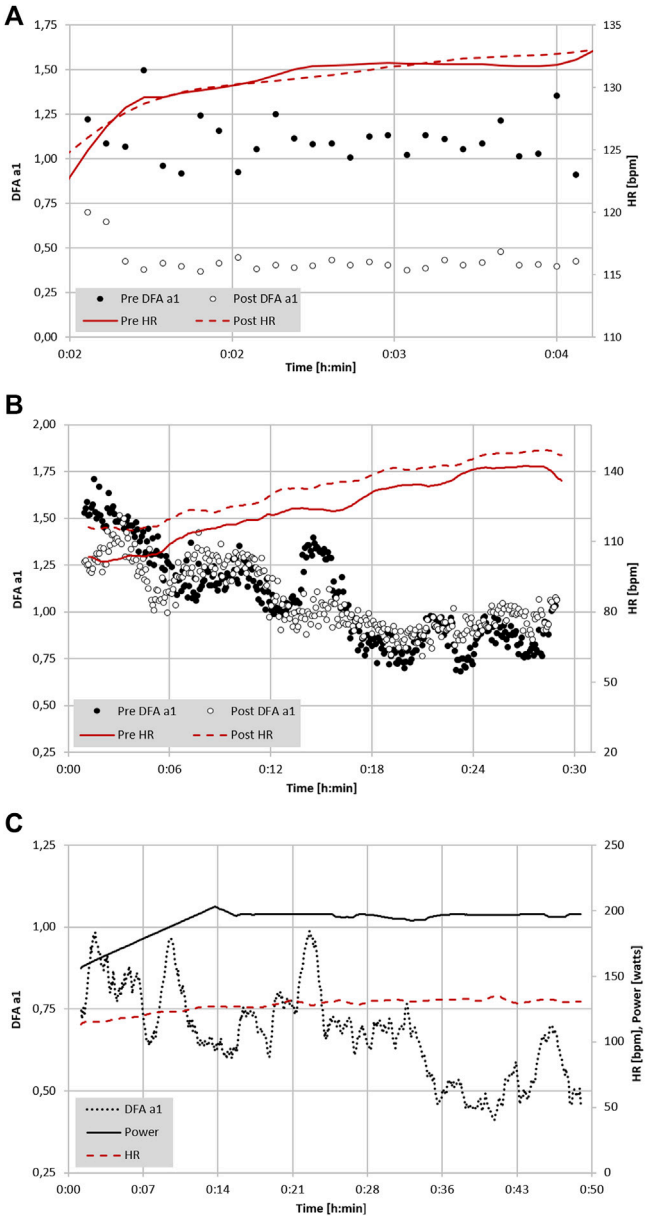

FIGURE 2 | (A) Analysis of DFA a1 and HR of a 22-year-old male participant with a VO_{2MAX} of 74 ml/kg/min during a 5-min treadmill test at 65% of VT1 (VO_2) before and immediately following a 6-h continuous trail run (data adapted from Rogers et al., 2021e). **(B)** Analysis of DFA a1, power and HR of a 41-year-old former Olympic male triathlete with a VO_{2MAX} of 65 ml/kg/min performing 6-min progressive cycling intervals of 100, 130, 160, 190, 220 W before and immediately after 2 h of continuous cycling exercise at 65% LT1 power (adapted from Gronwald et al., 2021). **(C)** Analysis of DFA a1 and HR of a 48-year-old male participant with a VO_{2MAX} of 46 ml/kg/min during 15 km cycling exercise at 90% of VT1 (VO_2) (unpublished data). All data recorded with a Polar H10 chest belt (Polar Electro Oy, Kempele, Finland) and analyzed with Kubios HRV Premium software Version 3.5 ("time varying" option: window width of 2 min, grid interval of 5 s); lines represent 4 points moving average.

h session performed at 65% of the LT1 power in a former Olympic triathlete (adapted from Gronwald et al., 2021). In the last example (**Figure 2C**), DFA a1 shows downward drift at an intensity just below the VT1 during a 15 km cycling session with stable HR in a recreational athlete. These three cases demonstrate the potential of DFA a1 to serve as an independent and systemic marker of fatigue and durability. The former Olympic triathlete showed no sign of DFA a1 "deterioration" after 2 h cycling at a low intensity, however the recreational athlete had declining DFA a1 at a stable power below the AeT, illustrating the potential interactions of inherent fitness level and external load over time.

In future studies looking at DFA a1 behavior at low to moderate intensities over longer time spans, incorporation of multipoint (rolling) averaging or longer measuring windows may lead to better comparative insights. Additionally, it will be important to examine the day-to-day variation and reproducibility of the DFA a1

versus external load relationship to determine what degree of precision can be expected for athletic assessments involving fatigue and durability.

LIMITATIONS AND PITFALLS

Effects of Artifact Correction

Although the state of research related to DFA a1 and endurance exercise is encouraging, there are still potential limitations for this approach. Both the common occurrence of missed beat artifact and possible RR recording device bias could lead to erroneous DFA a1 values. Previous studies looking at missed beat artifact correction bias indicated variable effects on DFA a1 and none examined how HRV based exercise thresholds could be changed with increasing artifact presence (Stapelberg et al., 2018). In addition, the popular software Kubios HRV has two distinct varieties of artifact correction methodology (automatic and threshold artifact correction; Lipponen and Tarvainen, 2019), whose potential influence with respect to DFA a1 calculation or HRVT determination should be evaluated. To better understand these issues, we examined the effect of introducing progressive amounts of dropped signal artifact (by randomly deleting QRS complexes) to induce levels of 1, 3 and 6% artifact in otherwise ideal ECG tracings (Rogers et al., 2021b). Both Kubios automatic and medium threshold artifact correction methods were evaluated for bias. A negligible amount of bias was produced by 1 and 3% artifact correction with both methods. A larger amount of proportional bias (positive bias at low DFA a1, negative bias at high DFA a1) was seen with the threshold correction method at the 6% artifact level, rising to a maximum of 19% bias at very low, anticorrelated values of DFA a1. Fortunately, despite the varying degrees of bias seen, the HR at the HRVT did not differ between control (no artifacts) and any of the artifact groups or correction methods by more than 1 bpm. This is certainly reassuring regarding practical usage of HRV based exercise threshold determination and other fields of application.

Recording Device Bias

Another aspect of the mentioned study about artefact correction (Rogers et al., 2021b) addressed whether the HRVT calculated from ECG was equivalent to the HRVT obtained by a chest belt (Polar H7) worn simultaneously. There was a small degree of bias at HRVT seen with the Polar H7, 4 bpm lower than that of the ECG. Several possibilities exist as to why there may be differences between devices. DFA a1 is a measure of RR related fractal correlation properties and therefore, specific patterns in the HR time series (Gronwald et al., 2020). Hence, a loss of RR resolution may lead to a failure to discern these patterns, leading to mistaken measures of fractal correlation properties. In support of this, a reduction in R peak detection precision has been shown to affect DFA a1 determination (Cassirame et al., 2019). According to Polar documentation, the R peak detection precision was enhanced in a next generation device, the Polar H10, implying that there was room for improvement in this property (Polar, 2019).

ECG and Chest Belt Sensor Placement

In addition to a purely device based lack of precision, RR measurement may also depend on which particular ECG lead is chosen for analysis. In an intriguing study, significant variation in both RR measurement and conventional HRV indexes was seen depending on ECG lead selection (Jeyhani et al., 2019). After searching for standards regarding device validation studies for RR interval detection, there appears to be a lack of consensus on ECG lead selection as to what constitutes the "gold standard" (Task Force, 1996; Sassi et al., 2015; Dobbs et al., 2019). Chest belt recordings are most similar to ECG lead 2 (Jeyhani et al., 2019), but certainly are not identical. Although this has not been examined for DFA a1, an example may shed light on this issue (see **Figure 3**). Depending on the particular ECG lead analyzed, slightly different DFA a1 values but only minimal differences in HRVT determination can be seen (based on DFA a1 crossing 0.75). It is also possible that even with equivalent monitoring precision and R peak detection, changing sensor pad placement can alter DFA a1 measurement to a variable degree. This is apparent in **Figure 3B** where there is some divergence in data points between ECG lead 2 and V3 and both chest strap devices. This dissimilarity may stem from person to person variation in the cardiac axis, leading to slight ECG waveform changes depending on the signal sampling location (Jeyhani et al., 2019). Further study into this subject may help optimize DFA a1 measurement.

Other Factors Affecting DFA a1 Behavior

From a network physiology standpoint, we must also keep in mind that DFA a1 is a reflection of net ANS activity and "organismic demand" of the systemic internal load. Therefore, changes in ambient temperature, fraction of inspired oxygen, intentional change of breathing frequency, hydration, nutritional status, and systemic illness can all play a role in modulating DFA a1 behavior. Lastly, though ANS balance appears to be the major determinant of DFA a1 behavior (Silva et al., 2015), locomotor-respiratory and cardiac coupling mechanisms and other non-neural mechanisms may also play a part (Persson, 1996; Qu et al., 2014). Finally, a major requisite of using pre-defined values of DFA a1 as a marker of exercise intensity is the need for uniform HRV software methodology in its calculation. One should also not expect exact similarity of results if different preprocessing methods from Kubios HRV are used for calculations (Voss et al., 2015).

NEW APPLICATIONS FOR DFA A1 COMPUTATION AND REAL-TIME DISPLAY

While DFA a1 is an appealing physiologic biomarker, important factors such as accurate, low cost calculation and simplified data display are needed for widespread consumer usage. Over the past year, several web and smartphone applications have been developed to display DFA a1 independently of analysis software such as Kubios HRV. Websites such as "Runalyze.com" and "AIEndurance.com" utilize similar preprocessing (smoothness priors detrending), artifact

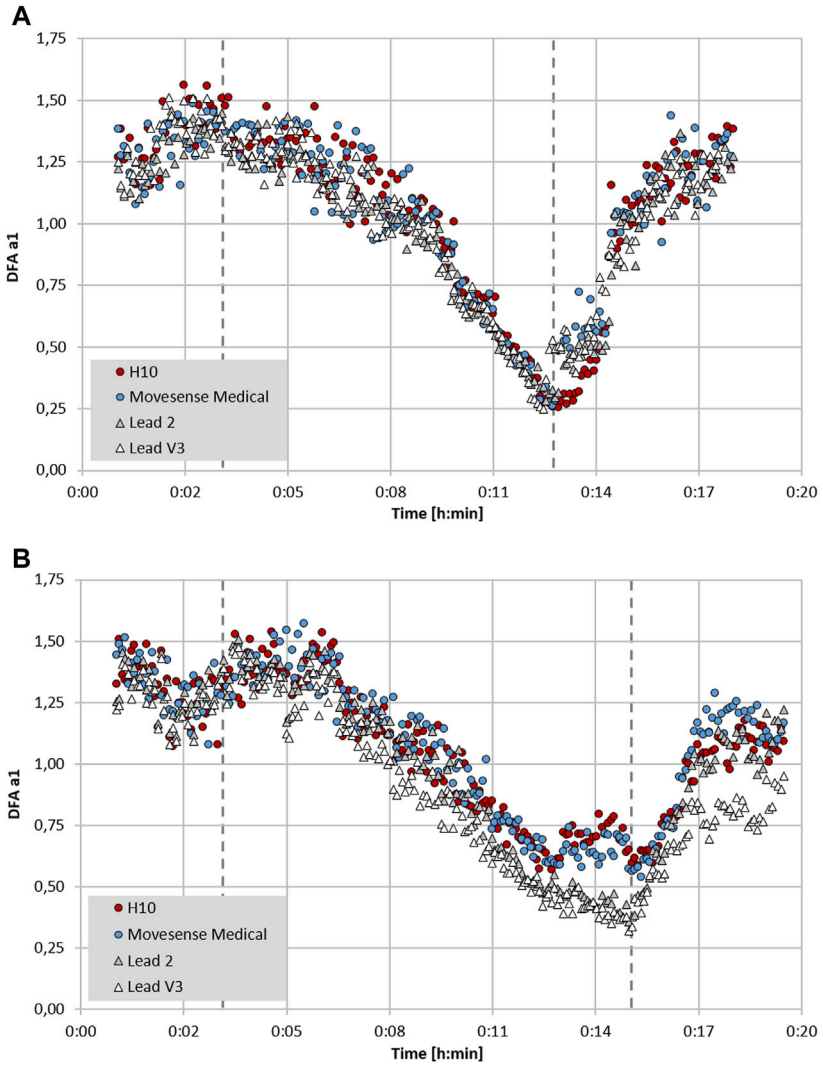

FIGURE 3 | Plot of DFA a1 over time during the course of an incremental cycling ramp test until voluntary exhaustion, including 3 min of warm-up at 50 W and 5 min cool-down of unloaded pedalling (dashed lines mark the start of the incremental test and voluntary exhaustion), with two participants wearing a 12 channel ECG CardioPart 12 Blue (AMEDTEC Medizintechnik Aue GmbH, Aue, Germany; sampling rate: 500 Hz), Polar H10 chest belt (Polar Electro Oy, Kempele, Finland; sampling rate: 1,000 Hz) and Movesense Medical sensor single channel ECG chest belt (Movesense, Vantaa, Finland; sampling rate: 512 Hz). **(A)** Participant 1 with similar DFA a1 seen in both chest belt devices and lead 2 and V3 of the 12 channel ECG. **(B)** Participant 2 with discrepancy between DFA a1 in both chest belt devices compared to lead 2 and V3 of the 12 channel ECG. All data analyzed with Kubios HRV Premium software Version 3.5 ("time varying" option: window width of 2 min, grid interval of 5 s) (data and plot adapted from Rogers et al., 2022b).

correction (threshold approach) and time varying DFA a1 calculation methodology as Kubios HRV Premium for retrospective session analysis of DFA a1. Standard Garmin.fit files can be uploaded for analysis with estimation of HRVT based on linear regression using time varying computation of power/HR and DFA a1. The AIEndurance platform also tracks previous warm-up session DFA a1 to power relationships to estimate "readiness to train" as well as overall session deterioration in DFA a1 per unit power for measures of "durability". Also of note is the availability of low cost smartphone apps that can process heart rate monitor data in real-time, providing a live view of DFA a1 status during exercise activities. A previous report described the use of the smartphone app "HRV Logger" (Android and iOS available) in both threshold determination and implementation of a polarized session program in a former Olympic triathlete (Gronwald et al., 2021). This particular application displays DFA a1 every 2 min sequentially without rolling window recomputation. A newer app called "Fatmaxxer" (Android available, https://github.com/IanPeake/FatMaxxer) enhances DFA a1 measurement capabilities even further. It includes features present in Kubios HRV Premium such as similar preprocessing (smoothness prior detrending), threshold correction and recalculation of DFA a1 e.g., every 5 s using 2-min rolling windows (time varying analysis: window width of 2 min, grid interval of 5 s). Lastly, "alphaHRV" a DFA a1 data field for Garmin devices (watches and cycling head units) is available in beta testing. It reports DFA a1 along with artifact percentage every

second in real-time based on the prior 200 beats, bringing this metric to potentially millions of Garmin units. Having the ability to display accurate DFA a1 in real-time during an exercise session opens up a myriad of potential options, from on the spot threshold determination to assessment of fatigue status at the start (for daily directed training) as well as during a long exercise session (durability assessment and/or training interventions).

FUTURE DIRECTIONS

A question that has eluded study thus far is whether DFA a1 behavior in male versus female participants is the same. Scant data exists at present showing HRVT similarity to the AeT in females as opposed to males. In addition, no information exists comparing DFA a1 response during exercise in pre- vs. post-menopausal women and along different phases of the menstrual cycle. From a theoretical standpoint, differences in DFA a1 response during exercise are possible in hormonally active women as several studies show altered HRV between the sexes (Abhishekh et al., 2013; Kappus et al., 2015). More specifically, autonomic balance appears to change over the course of the menstrual cycle (Brar et al., 2015) as opposed to a lack of change in cardiorespiratory parameters (Rael et al., 2021). An additional area of interest is HRVT validity in other sport-specific settings, especially regarding the influence of upper body activity on signal quality and differences in DFA a1 calculation (e.g., skiing, swimming, rowing), and over diverse age groups which would be helpful for widespread usage. In addition, consequences of intermittent positional bounce such as seen with running could be examined since they do have the potential to alter the cardiac axis, thereby affecting ECG waveform morphology (Astrom et al., 2003). Another question is whether the improvement of DFA a1 per unit of power or pace after a training intervention can indicate enhanced fitness, particularly in threshold boundaries. Preliminary data is suggestive (Rogers et at., 2021f), but additional research is needed. Prospective training intervention studies of participants who utilize knowledge of DFA a1 behavior during the early stage of routine exercise sessions for training guidance (as shown in monitoring with time domain HRV values during rest conditions (Düking et al., 2021)) would be of great interest as a tool to determine daily "readiness to train".

CONCLUSION

Studies of fractal correlation properties of heart rate variability during exercise have produced important findings over the past two years. DFA a1 behavior is a reflection of ANS regulation and is a component of network physiology which encompasses multiple neuromuscular, biochemical, peripheral and central nervous system inputs leading to an overall index of "organismic demand". A distinct DFA a1 value of 0.75 has been shown to correspond to the aerobic threshold in recreational runners, elite triathletes and patients with cardiac disease. Further DFA a1 decline to the 0.5 range, signifying an uncorrelated random beat pattern, was associated with the anaerobic threshold, with both concepts representing limits of sustainable workloads. DFA a1 threshold determination can be affected by artifact correction and recording device bias but under most circumstances has enough resiliency to be of practical value with consumer grade heart rate monitors used for RR interval detection. Exercise modalities associated with difficulty in assessing intensity such as eccentric cycling may benefit from further investigation of DFA a1 as a marker of systemic internal load. Both web based and real-time smartphone tracking apps have been developed for DFA a1 monitoring and physiologic exercise threshold determination. Many of the current applications use similar computational methodology as the industry analysis standard of Kubios HRV software. Finally, while of value when even viewed in isolation, DFA a1 tracking in combination with external load markers such as power or pace open intriguing possibilities regarding athlete durability, identification of endurance exercise fatigue and optimization of daily training guidance.

AUTHOR CONTRIBUTIONS

TG and BR conceived the study. BR and TG performed the data analysis and wrote the first draft of the article. All authors (BR, TG) revised it critically for important intellectual content, final approval of the version to be published, and accountability for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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The Six-Minute Stepper Test Is Valid to Evaluate Functional Capacity in Hospitalized Patients With Exacerbated COPD

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Background: The six-minute stepper test (6MST) is a self-paced test considered a valid tool to assess functional capacity in stable COPD patients. However, a high floor effect, where a large proportion of participants reach the minimum score when using the measurement instrument, might compromise the test validity in the hospital setting. Therefore, this study aimed at verifying the concurrent validity of 6MST in hospitalized patients with acute exacerbation of COPD (AECOPD).

Methods: A cross-sectional study was conducted in a tertiary hospital. Patients who were hospitalized due to AECOPD were considered for inclusion. On the first day, when patients reached minimum clinical criteria considered as the use of non-invasive ventilation less than 2 h for 6 h/period, dyspnea at rest less than 7 (very severe) on the modified Borg scale, a respiratory rate less than 25 breaths per minute, oxygen pulse saturation greater than 88% (considering use of supplemental oxygen) and absence of paradoxical breathing pattern, they underwent a lung function evaluation and answered three questionnaires: Chronic Respiratory Questionnaire (CRQ), Modified Medical Research Council Dyspnea Scale (MMRC), and COPD Assessment Test (CAT). Then, on two consecutive days, patients performed 6MST or six-minute walk test (6MWT), in random order. Each test was performed twice, and the best performance was recorded. Also, the patient's severity was classified according to the BODE index. Inspiratory capacity measurements were performed before and after each test execution.

Results: Sixteen patients (69.4 ± 11.4 years) with a mean FEV_1 of 49.4 ± 9.9% predicted were included (9 females). There was a strong correlation of the performance in 6MST (number of cycles) with 6MWT (distance walked in meters) in absolute values ($r = 0.87$, $p < 0.001$) as well as with the percentage of predicted normal 6MWT ($r = 0.86$, $p < 0.001$). There was a strong correlation between the performance in 6MST with the dynamic hyperinflation ($r = 0.72$, $p = 0.002$) and a moderate correlation between 6MST with the

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Abbreviations: COPD, chronic obstructive pulmonary disease; 6MST, six-minute stepper test; AECOPD, acute exacerbation of COPD; CRQ, Chronic Respiratory Questionnaire; MMRC, Modified Medical Research Council Dyspnea Scale; CAT, COPD Assessment Test; 6MWT, six-minute walk test; COSMIN, COnsensus-based Standards for the selection of health Measurement INstruments; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in the first second; VC, vital capacity; IC, inspiratory capacity; ATS, American Thoracic Society; ERS, European Respiratory Society; DH, dynamic hyperinflation; RR, respiratory rate; HR, heart rate; SD, standard deviation.

percentage of reduction of inspiratory capacity (r = 0.68, $p = 0.004$). We also identified that 6MST showed moderate negative correlations with CAT (r = –0.62, $p = 0.01$) and BODE index (r = –0.59, $p = 0.01$).

Conclusion: It could be concluded that 6MST is valid for evaluating functional capacity in hospitalized patients with exacerbated COPD.

Keywords: COPD, exacerbation, physical activity, exercise capacity, dynamic hyperinflation

INTRODUCTION

COPD is one of the leading causes of morbidity and mortality worldwide, resulting in a substantial and growing economic and social impact (Lopez et al., 2006). Exacerbations of COPD are directly associated with worsening quality of life (Vestbo et al., 2013), accelerated decline in lung function (Garcia-Aymerich et al., 2011), reduced exercise capacity (Pitta et al., 2006), reduced daily living activities (Pitta et al., 2006), a significant increase in mortality (especially in patients requiring hospitalization) (Garcia-Aymerich et al., 2011), and increased socioeconomic costs (Vestbo et al., 2013). Exacerbated COPD patients have an amplification of the reduced exercise capacity characteristics of the disease (Pitta et al., 2006). In addition to ventilatory impairment, which is the main limitation of exercise capacity, other factors can also contribute to this reduction, such as peripheral muscle weakness (Choudhury et al., 2014). Several mechanisms can contribute to the skeletal muscle weakness found during acute exacerbation (Spruit et al., 2003; Pitta et al., 2006), such as the presence of systemic inflammation (Spruit et al., 2003), nutritional changes (Creutzberg et al., 2000), administration of oral corticosteroids (Decramer et al., 1994), and a sedentary lifestyle (Pitta et al., 2006).

Even with the appropriate adjustment of pharmacological therapy, the presence of high morbidity and mortality, as well as the persistent intolerance to physical exercise that occurs in patients with COPD, justify the need for research on new treatment strategies, such as regular and continuous physical training, based mainly on the patient's functional capacity. Furthermore, it is known that functional capacity is impaired in this profile of patients, and may present progressive worsening during hospitalization, thus, the correct assessment of functionality becomes essential, contributing positively to the planning of individually designed therapeutic strategies, optimization of prescription of exercises, in addition to monitoring the evolution of their functionality and clinical condition, as well as the responses and progress of rehabilitation programs in hospitalized patients. An established and widely used way to assess the functional capacity of these patients is the 6-min walk test (6MWT). However, although the 6MWT is a simple field test, well adapted and validated in several populations, a low-cost and widely feasible test, it has some disadvantages. Perhaps the main one is the need for a corridor to perform the walk with at least 30 m, to represent a valid test, as recommended by the American Thoracic Society and European Respiratory Society (Holland et al., 2014). Thus, to circumvent this 6MWT disadvantage, especially in the hospital environment, the assessment of functional capacity can be performed using other tests, such as the step test (José and Dal Corso, 2016), the Four-meter gait speed (Kon et al., 2013), the Timed "Up and Go" (Johnston et al., 2017), as well as the six-minute stepper test (6MST) (Borel et al., 2010).

The 6MST uses a stepper, a portable device that simulates the climbing of steps, moving with the individual's action, eliminating the need for an extensive corridor to perform the assessment. The 6MST is a self-paced test, like the 6MWT, and its primary outcome is the number of cycles completed in 6 minutes. The 6MST is considered a feasible technique for assessment of functional capacity in patients with different diseases such as COPD (Coquart et al., 2014), interstitial lung diseases (Delourme et al., 2012), older adults (Jones et al., 2017), home-based pulmonary rehabilitation (Grosbois et al., 2019a; Grosbois et al., 2020), asthma (Grosbois et al., 2019b), fibrotic idiopathic interstitial pneumonia (Wallaert et al., 2019), and chemotherapy-treated patients with thoracic cancers (Olivier et al., 2018), as an alternative to the 6MWT. Furthermore, in a population of hospitalized and healthy older people, the 6MST showed convergent validity with the functional variables used to diagnose sarcopenia (Francisco et al., 2020). 6MST is a submaximal test, reproducible and well-tolerated assessment tool. In addition, it is an inexpensive and portable method to assess exercise tolerance in patients with stable COPD (Borel et al., 2010). In a previous study, 6MST was applied to circumvent the environmental restrictions of the 6MWT, and significant correlations were observed between the 6MST and the 6MWT in oxygen consumption and heart rate (Borel et al., 2010). That same study also suggested discriminative properties of the test, as it found significantly higher performance in healthy individuals compared to patients with COPD.

Furthermore, another study was also able to demonstrate the sensitivity of the 6MST to detect improvement in functional capacity after pulmonary rehabilitation in patients with COPD (Coquart et al., 2014). In addition, another relevant characteristic of 6MST is that it allows the prescription of training for patients with COPD (Bonnevie et al., 2017). Although the 6MST has been validated in stable patients, the possibility of a high floor effect could compromise the validity of this tool in the hospital setting, that is, individuals who reach the minimum score when using the 6MST due to the possible limitations of their clinical condition. Thus, this study aimed at verifying the concurrent validity of 6MST to assess the functional capacity in hospitalized patients with acute exacerbation of COPD (AECOPD).

Our study was based on the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) and its checklist (Mokkink et al., 2010), for analysis of the methodological quality of the concurrent validation assessment (COSMIN box H. Criterion Validity). Criterion Validity is the degree to which the scores of a measuring instrument are an

adequate reflection of a gold standard (Scholtes et al., 2011), in our case, the 6MWT.

PATIENTS AND METHODS

Ethics and Participants

The sample was obtained consecutively, recruiting patients of both sexes admitted to the Hospital Sírio-Libanês for treatment of AECOPD. The study included individuals who met the following criteria: 1) patients with a previous medical diagnosis of COPD before hospitalization; 2) diagnosis of exacerbated COPD classified as level II (Celli et al., 2004b), 3) absence of cognitive or motor deficit that limited the execution of the tests; 4) absence of previous cardiovascular disease; 5) no previous thoracoabdominal surgery within 1 month; 6) body mass index (BMI) < 30 kg/m^2; and 7) no use of vasoactive drugs. The exclusion criteria were considered: 1) inability to perform the evaluations within the criteria of technical acceptability and 2) cardiorespiratory instability during the tests (severe dyspnea, arrhythmias, angina, elevated heart rate above 80% of maximum heart rate, and oxygen pulse saturation below 88% refractory to oxygen supplementation). The study was previously approved by the research ethics committee of the Hospital Sírio-Libanês (approval protocol 3.432.823) and written informed consent was provided by all participants.

Study Design and Experimental Procedures

In the cross-sectional design of this study, the patients underwent an evaluation protocol performed on two consecutive days. The protocol was applied from the moment that the patients had the following minimum clinical criteria: use of non-invasive ventilation less than 2 h per 6-h/period, dyspnea at rest less than 7 (very intense) on the modified Borg scale, respiratory rate less than 25 incursions per minute, oxygen pulse saturation greater than 88% (considering supplemental oxygen use) and absence of paradoxical breathing pattern. On the first day of the evaluation, the subjects answered three specific evaluation questionnaires related to lung disease: 1) the Chronic Respiratory Questionnaire (CRQ), 2) the modified Dyspnea Scale of the Medical Research Council (MMRC), and 3) the COPD Assessment Test (CAT). In addition, anthropometry, pulmonary function tests, and two evaluations of 6MST and 6MWT (in randomized order by closed envelopes) were performed. Thirty minutes after the execution of the first test, the same test selected was conducted again. On the second day, individuals underwent the other exercise tolerance test (6MST or 6MWT, as randomized), which was also repeated after 30 min. Each test was carried out twice and the best performance was recorded. Also, the patient's severity was classified according to the BODE index. Inspiratory capacity measurements were obtained before and after each test execution.

The pulmonary function test was performed using a portable spirometer (Koko pulmonary function testing model; nSpire Health Company, Longmont, CO, United States), previously calibrated according to the methods and criteria recommended by the American Thoracic Society (Miller et al., 2005). At least three acceptable maneuvers and two repeatable maneuvers were performed. The highest values obtained for each of the spirometric variables were considered, which were expressed in absolute and in the percentage of the expected values of normality (Pereira et al., 2007). Post-bronchodilator spirometry measurements were performed to assess the response to drug therapy. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), FEV_1/FVC ratio, vital capacity (VC), and inspiratory capacity (IC) were assessed.

The CRQ has been widely used in the analysis of the health status of patients with COPD (Moreira et al., 2009). This questionnaire contains 20 questions, divided into four domains: dyspnea, fatigue, emotional function, and self-control. A higher score achieved is associated with a better quality of life for the subject.

A widely used tool to assess the effect of dyspnea on activities of daily living is the MMRC scale. It consists of a 5-item questionnaire in which patients categorize their degree of disability, reflecting how dyspnea affects their mobility (Wedzicha et al., 1998; Bestall et al., 1999). The patients report their subjective degree of dyspnea, choosing a value between 0 and 4. Lower scores in the MMRC are associated with less impairment of activities of daily living related to dyspnea.

The CAT questionnaire was used to assess the clinical impact of COPD symptoms. This tool has the characteristics of being a short and simple instrument to quantify the impact of COPD symptoms on clinical practice, assist in assessing health status, and facilitate communication between patients and the health professional (Jones et al., 2012). This questionnaire has been validated for the Brazilian population with COPD (Silva et al., 2013). It consists of eight items: cough, phlegm, chest tightness, shortness of breath, limitations in-home activities, confidence in leaving home, sleep, and energy. Results vary according to the score range obtained, classified as follows concerning clinical impact: 6–10 points, mild; 11–20, moderate; 21–30, severe; and 31–40, very severe.

The BODE index serves as a predictor of mortality risk that assesses individuals with COPD systemically (Celli et al., 2004a; Araujo and Holanda, 2010). Furthermore, it is also used to classify the severity of COPD, determine the risk of hospitalizations due to exacerbation (Ong et al., 2005), and predict response to pulmonary rehabilitation programs (Cote and Celli, 2005). Its assessment includes the body mass index (BMI), the degree of obstruction to the expiratory flow through FEV_1, the perception of dyspnea using the MMRC scale, and the exercise tolerance, assessed by the performance in the 6MWT. However, its greater use and importance stems from the fact that it does not evaluate the degree of airway obstruction in isolation. It analyzes COPD's respiratory and systemic manifestations and can better characterize and predict outcomes in this population (Da Silva et al., 2013).

The 6MWT was performed in a flat corridor 30 m long and 1.5 m wide, previously marked. The individuals were instructed and encouraged to walk as far as possible for 6 minutes, using standardized incentive phrases every minute, as recommended by the American Thoracic Society (ATS) and European Respiratory Society (ERS) (Holland et al., 2014). The 6MWT was performed twice on the same day, with an interval of 30 min between tests. For the analysis, the longest distance presented between the two tests was used. The predicted values were obtained from the reference equation proposed by Britto and colleagues (Britto et al., 2013). Inspiratory capacity (IC), oxygen pulse saturation, respiratory rate

FIGURE 1 | Pictures of the stepper and position adopted during the six-minute stepper test.

(RR), heart rate (HR), symptoms of dyspnea and lower limb fatigue (verified by the modified Borg scale), and blood pressure measurements were checked at rest and immediately after the test. Pulmonary hyperinflation, defined as the difference between the IC measured in the pre-and post-test period, was considered present when a 10% and/or 150 ml reduction in the post-test IC was demonstrated concerning the pre-test IC (O'Donnell et al., 2001; Colucci et al., 2010). All measurements in both tests (6MWT and 6MST), were evaluated with the patient seated upright, with feet flat on the floor and legs uncrossed. Measurements at the end of the test were performed as soon as the test ended and the patient assumed the sitting position. Two evaluators performed data collection and measurements. The inspiratory capacity (IC) was assessed using a portable spirometer (Koko pulmonary function testing model; nSpire Health Company, Longmont, CO, United States). The IC measurement maneuver was performed after the stabilization of the end-expiratory lung volume verified by the equipment. The patient was encouraged to perform a maximal voluntary inspiratory maneuver for TLC both during the evaluation at rest and at the end of the exercise. The oxygen pulse saturation and heart rate (HR) were assessed using a portable pulse oximeter (pulse oximeter Nonin; Nonin Medical Inc., Plymouth, MN, United States) and the blood pressure measurements were checked using a sphygmomanometer (sphygmomanometer Omron, Omron Healthcare Brasil, Jundiaí, SP, Brazil). One evaluator performed respiratory rate counting and questioned the patient about dyspnea and lower limb fatigue symptoms. The other evaluator assessed the oxygen pulse saturation, heart rate, blood pressure, and inspiratory capacity measurements.

The 6MST, using the stepper (Mini Stepper, Mor, Santa Cruz do Sul, RS, Brazil), was performed in an isolated room to avoid any stimulus that could influence the performance of the activity. The stepper used had a support rod to allow the patients to support themselves when they were unbalanced or exhausted. The initial position of the stepper was as follows: right or left foot, according to the individual's choice, in the elevated position and the other foot in the lower position, with the arms along the body (**Figure 1**).

The adjustment of the height of the elevated position of the stepper was 20 cm. The test was performed after the patient's familiarization with the equipment, remaining at rest in the initial position for a period of up to 2 minutes, if necessary. The 6MST followed the same ATS/ERS recommendations for the 6MWT, using the same standardized incentive phrases every minute. Patients were instructed and encouraged to perform the full cycle of the device as many times as possible in 6 minutes.

The full cycle is defined as the return to the starting position (Borel et al., 2010). The value provided by the device's digital display was used to count the total number of cycles performed in the stepper. The equipment display was positioned opposite the patient, making it impossible for the participant to see the step count. 6MST was performed twice on the same day, with an interval of 30 min between tests. The number of full cycles performed in the tests was used for the analysis. As with the 6MWT, measurements of IC, oxygen pulse saturation, respiratory rate, heart rate, symptoms of dyspnea and lower limb fatigue, and blood pressure were checked at the same time as the test was performed.

Statistical Analyses

The GraphPad Prism 6 Statistical Package (GraphPad Software, San Diego, CA, United States) was used for the statistical analysis of the data, presented by the mean and standard deviation (SD). The normality of the data was assessed using the Shapiro Wilk test. To evaluate the correlation of the performance in 6MST with the performance in 6MWT, dynamic hyperinflation, questionnaires, and specific index associated with lung disease (MMRC, BODE, CAT, and CRQ), spirometric variables (FEV_1, FEV_1/FVC, FVC, and baseline IC), and variations of vital signs (oxygen pulse saturation, HR, systolic and diastolic blood pressure, and RR) and the perception of dyspnea and fatigue of lower limbs before and after testing, and between the variations magnitude of vital signs and perception of dyspnea and fatigue of lower limbs from rest during the 6MST and 6MWT, Pearson's correlation test (parametric data) or Spearman's correlation test (nonparametric data) were used. The magnitude of the correlations was based on Munro's classification (Munro, 2001): none or small, from 0 to 0.25; weak from 0.26 to 0.49; moderate, from 0.50 to 0.69; strong, from 0.70 to 0.89; very strong, from 0.90 to 1.00. The performances of the two 6MST tests were compared for repeatability analysis using the paired Student's t-test (parametric data). Repeatability is considered the variation of the response depending on the same measuring device and evaluator; therefore, repeatability refers to the performance of serial measurements under the same conditions, performed over a short time, by the same evaluator using the same equipment. For all tests, a $p < 0.05$ was considered statistically significant. According to the COSMIN guidelines, it is possible to perform sample size calculations for expected correlations between measures in validity studies. The

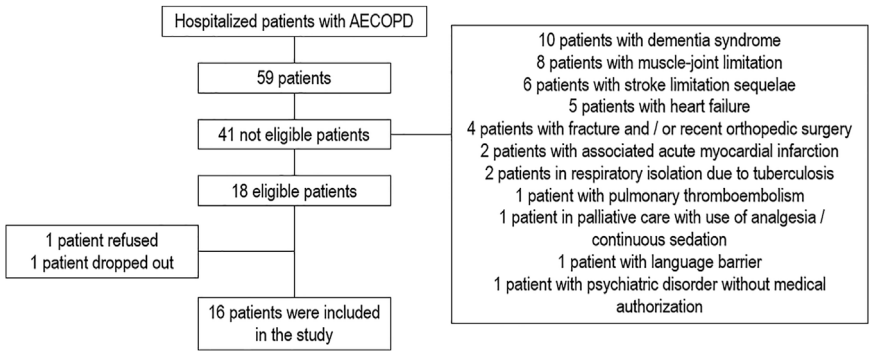

FIGURE 2 | Screening flowchart for hospitalized patients with AECOPD. Abbreviations: AECOPD, acute exacerbation of COPD.

TABLE 1 | Characterization of the study participants (n = 16).

Demographic and anthropometric data	
Age (years)	69.4 ± 11.4
Sex (M/F)	7/9
BMI (kg/m^2)	23.7 ± 4.7
Clinical characteristics	
GOLD stages (II/III)	8/8
Length of hospital stay (days)	8.6 ± 4
Evaluation day (day)	4.5 ± 1.9
Use of oxygen therapy (%)	31.2
NIV use for a period ≥1 h/6 h (%)	50
Smoker/ex-smoker	10/6
Smoking load (pack years)	59.1 ± 23.4
Comorbidities	
Systemic arterial hypertension (%)	62.5
Diabetes (%)	18.8
Dyslipidemia (%)	31.3
Depression (%)	25
Hypothyroidism (%)	6.3
Obstructive sleep apnea syndrome (%)	6.3
Pulmonary function	
FEV_1 (% predicted)	49.4 ± 9.9
FVC (% predicted)	77.3 ± 15.2
FEV_1/FVC	0.5 ± 0.1
IC (liters)	1.7 ± 0.4
Questionnaires data	
MRC modified (score)	1.6 ± 1.3
BODE (score)	3.6 ± 2.2
CAT (score)	15 ± 5.3
CRQ—dyspnea (score)	3.3 ± 1
CRQ—fatigue (score)	3 ± 1.1
CRQ—emotional function (score)	4 ± 1.1
CRQ—self-control (score)	4.5 ± 1.5

Notes: Data are presented as mean ± SD. FEV₁/FVC, the ratio of FEV_1 to FVC; M = male; F = female; BMI, body mass index; Kg/m^2 = kilograms per square meter; GOLD, global initiative for chronic obstructive lung disease; Y = yes; N = not; NIV, non-invasive ventilation; FEV_1 (predicted%) = percentage of predicted for the forced expiratory volume in the first second; FVC (% predicted) = percentage of predicted for forced vital capacity; FVC/FEV_1 = ratio of forced vital capacity to forced expiratory volume in the first second; IC, inspiratory capacity; Modified MRC, medical research council modified dyspnea scale; BODE = BODE, index (Body mass index, Airway Obstruction, Dyspnea, and Exercise capacity); CAT = COPD, assessment test; CRQ, chronic respiratory questionnaire.

6MST and 6MWT instruments have continuous scores. Therefore, the preferred method is the presentation of the correlation coefficient. This correlation should preferably be above 0.70 (COSMIN box H-10).

TABLE 2 | Performance average in first, second, and the best 6MWT and 6MST.

	6MWT (First test)	6MWT (Second test)	6MWT (Best test)
Performance (meters)	279.3 ± 90.8	300.6 ± 108.5	310 ± 96.2
Performance (%predicted)*	53.6 ± 15.2	59 ± 16.9	59.6 ± 16.7
	6MST (First test)	**6MST (Second test)**	**6MST (Best test)**
Performance (cycles)	141.8 ± 55.2	155.6 ± 62.9	159.8 ± 59.9

*Notes: Data are presented as mean ± SD., 6MWT, 6-min walk test; 6MST, 6-min stepper test. * The derived equation for both genders was: 6MWT, distance = 356.658– (2,303 × age) + (36,648 × gender) + (1,704 × height) + (1,365 × Δheart rate). When male gender = 1 and female gender = 0 (Britto et al., 2013).*

Based on the results of a previous study (Pichon et al., 2016) which found a significant correlation (r = 0.72) between the number of steps during 6MST and the 6MWT pre-pulmonary rehabilitation, and estimating a similar effect, using an error of 5% (power test time 90%), the need to include 16 patients in the study was calculated (COSMIN box H-3).

RESULTS

A total of 59 hospitalized patients with a diagnosis of exacerbated COPD were screened, among which 41 patients did not meet the inclusion criteria. In total, 18 patients met the inclusion criteria; however, 1 patient refused, and 1 patient dropped out on the second day of evaluation (**Figure 2**) (COSMIN box H-5). Thus, a total of 16 patients were included in the study, being 9 female (56.25%) and 7 male (43.75%), with a mean age of 69.38 ± 11.42 years and FEV_1 of 49.38 ± 9.86% of the predicted. According to the GOLD classification, 8 patients (50%) were categorized as GOLD II, and 8 patients (50%) as GOLD III. These results are described in **Table 1**. The results of the performance average in the first, second, and the best 6MWT and 6MST; and physiological and perceptual parameters at rest and the end of the best tests performed are described in **Tables 2**, **3**, respectively. There was no data loss for any variables (COSMIN box H-1 and H-2).

TABLE 3 | Physiological and perceptual parameters at rest (initial) and the end (final) of the best tests (6MWT and 6MST) performed.

6MWT (best test)	Initial	Final	Difference	Difference (%)
IC (liters)	1.7 ± 0.4	1.5 ± 0.4	–0.2	–11.8
SpO_2 (%)	94.9 ± 2.8	92.4 ± 2.4	–2.4	–2.5
HR (bpm)	80.6 ± 12.3	96.6 ± 18.7	15.9	19.7
RR (bpm)	19.1 ± 3.6	21.9 ± 4.4	2.8	14.7
SBP (mmHg)	127.2 ± 7.7	143.8 ± 10.9	16.6	13.1
DBP (mmHg)	80.9 ± 8.4	83.4 ± 9.1	2.5	3.1
Borg Dyspnea	0.6 ± 1.1	1.1 ± 1.6	0.5	83.3
Borg Fatigue	0.4 ± 0.8	1.5 ± 1.7	1.1	275
6MST (best test)	**Initial**	**Final**	**Difference**	**Difference (%)**
IC (liters)	1.7 ± 0.4	1.5 ± 0.4	–0.2	–11.8
SpO_2 (%)	94.3 ± 3	94.6 ± 3.7	0.3	0.3
HR (bpm)	84.6 ± 16.3	110.2 ± 20	25.6	30.3
RR (bpm)	18.9 ± 3.6	24.1 ± 5.6	5.3	28
SBP (mmHg)	121.6 ± 10.8	147.5 ± 23.2	25.9	21.3
DBP (mmHg)	72.8 ± 9.1	82.5 ± 14.6	9.7	13.3
Borg Dyspnea	0.1 ± 0.5	2.9 ± 2.9	2.8	2,900
Borg Fatigue	0.4 ± 1	5.1 ± 2.5	4.7	1,175

Notes: Data are presented as mean ± SD., 6MWT, 6-min walk test; 6MST, 6-min stepper test; IC, inspiratory capacity; SpO2, oxygen saturation; HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

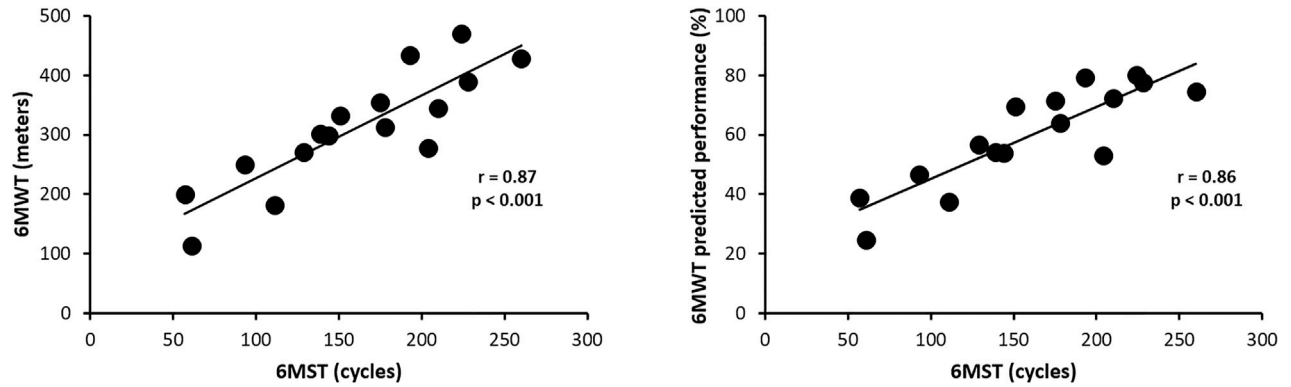

FIGURE 3 | Correlation of performances in 6MST and 6MWT in absolute values and predicted values. Abbreviations: 6MST, 6-min stepper test; 6MWT, 6-min walk test.

CONCURRENT VALIDITY

Relationship of Performance on 6MST With Performance on 6MWT

The performance of individuals in the 6MST showed a strong correlation both with the absolute value (r = 0.87; $p < 0.001$) and with the predicted value (r = 0.86; $p < 0.001$) in the 6MWT (**Figure 3**).

Relationship of Performance on 6MST With Dynamic Hyperinflation (Absolute and Percentage)

There was a strong statistically significant correlation between 6MST performance and dynamic hyperinflation (DH) development in absolute values (r = 0.72; $p = 0.002$), and a statistically significant moderate correlation between 6MST performance and DH development in percentage values (r = 0.68; $p = 0.004$) (**Figure 4**).

Relationship of Performance on 6MST With Questionnaires and Specific Indexes Associated With Lung Disease (Modified MRC, BODE, CAT, and CRQ)

There was a statistically significant moderate correlation between performance in 6MST and the score achieved in CAT (r = 0.62; $p = 0.01$) and BODE (r = 0.59; $p = 0.01$) (**Figure 5**). There was no statistically significant correlation between performance in 6MST and the score achieved on the modified MRC scale ($p = 0.11$), as well as in CRQ, in all domains: dyspnea ($p = 0.19$), fatigue ($p = 0.19$), emotional function ($p = 0.96$) and self-control ($p = 0.30$).

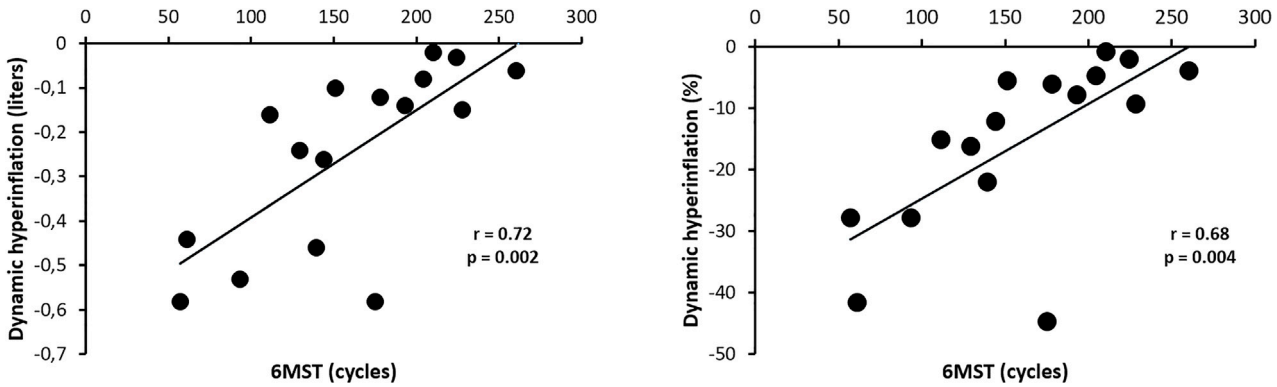

FIGURE 4 | Correlation of 6MST performance with the dynamic hyperinflation in absolute values and percentage. Abbreviations: 6MST, 6-min stepper test.

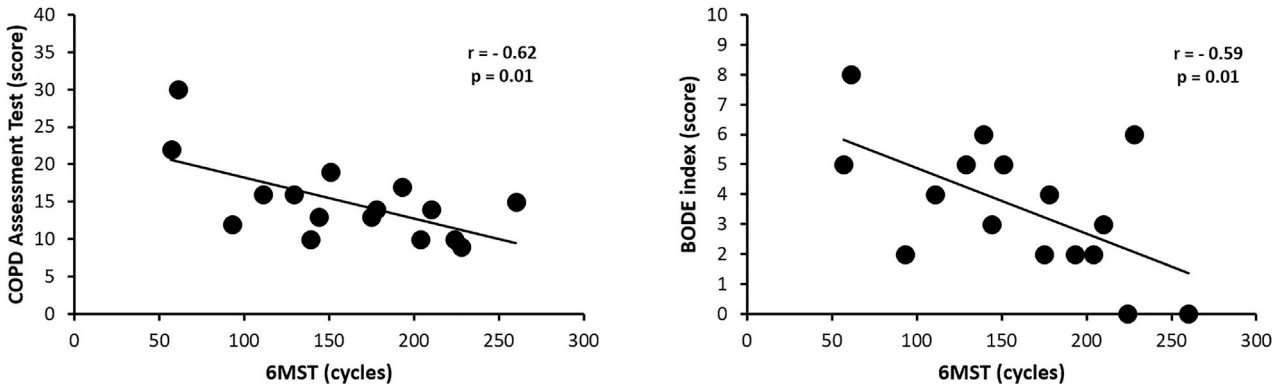

FIGURE 5 | Correlation of 6MST performance with the COPD Assessment Test and the BODE index. Abbreviations: 6MST, 6-min stepper test.

Relationship of Performance on 6MST With Spirometric Variables (FEV_1, FEV_1/FVC, FVC, and Baseline IC)

There was no statistically significant correlation between 6MST performance and FEV_1 ($p = 0.29$), FEV_1/FVC ($p = 0.50$), FVC ($p = 0.33$) and baseline IC ($p = 0.68$).

Relationship of Performance on 6MST with Variations in Vital Signs (Oxygen Pulse Saturation, HR, Systolic and Diastolic Blood Pressure, and RR) and the Perception of Dyspnea and Fatigue of Lower Limbs Before and After Testing

There was no statistically significant correlation between performance in 6MST and variations in both absolute and percentage of oxygen pulse saturation ($p = 0.79$ and 0.78, respectively), HR ($p = 0.12$ and 0.09, respectively), systolic blood pressure ($p = 0.27$ and 0.31, respectively), diastolic blood pressure ($p = 0.55$ and 0.46, respectively), RR ($p = 0.10$ and 0.33, respectively), and the absolute value of perception of dyspnea ($p = 0.65$) and lower limb fatigue ($p = 0.97$).

Relationship Between the Variation's Magnitude of Vital Signs (Oxygen Pulse Saturation, HR, Systolic and Diastolic Blood Pressure, and RR) and Perception of Dyspnea and Fatigue of Lower Limbs fromRest During the 6MST and 6MWT

There was a statistically significant strong correlation between the magnitude of variation of respiratory rate (r = 0.77; $p < 0.001$) and moderate correlation between the magnitude of variation of perception of dyspnea (r = 0.50; $p = 0.04$) from rest during the 6MST and 6MWT. There was no statistically significant correlation between the magnitude of variation of IC ($p = 0.17$), oxygen pulse saturation ($p = 0.34$), HR ($p = 0.14$), systolic blood pressure ($p = 0.06$), diastolic blood pressure ($p = 0.07$), and the perception of lower limb fatigue ($p = 0.8$) from rest during the 6MST and 6MWT.

Repeatability

The average performance on the first 6MST performed was 141.81 cycles, and on the second 6MST, it was 155.63 cycles,

which means a difference of 13.82 cycles, with no statistical significance (p = 0.0575).

DISCUSSION

The current study aimed to verify the validity of 6MST for the assessment of functional capacity in hospitalized patients with AECOPD. Our results demonstrated the concurrent validation of 6MST with the 6MWT to evaluate functional capacity in this setting. A pioneering study of 6MST already suggested the concurrent validation of the test concerning the 6MWT; however, the population of this study was composed of individuals with COPD in a phase of clinical stability (Borel et al., 2010). Also, in this population of patients with stable COPD, enrolled in a pulmonary rehabilitation program, the concurrent validation of 6MST with the 6MWT was demonstrated by correlation, in addition to the distance covered in the 6MWT, both power and oxygen consumption at maximum effort (Wallaert et al., 2016). Besides that, other authors found a significant correlation between performance in 6MST with the distance covered in the 6MWT and oxygen consumption in frail elderly individuals (Jones et al., 2017), in a sample with a mean age of 71.2 years, a value close to the average age of the sample of our study (69.38 years).

Likewise, the present study suggested a correlation through the 6MST performance with the ventilatory responses (absolute value and percentage of DH), the degree of severity and mortality risk of the individual with COPD (BODE), and the impact of the disease on quality of life (CAT). Our results agree with a previous study (Marin et al., 2001), which also showed the development of DH in patients with COPD when they performed the 6MWT. These authors also found a statistically significant correlation between the final IC and performance on the 6MWT. Another study (Satake et al., 2015), in addition to correlating the development of DH with the distance covered in the 6MWT in individuals with COPD, also indicated a significant negative correlation between the decrease in IC and the increase in dyspnea after the 6MWT. To our knowledge, no study has analyzed the correlation between DH and 6MST. Regarding the degree of severity and mortality risk associated with COPD (BODE), the results of the present study suggest that the greater the impairment of this index, the worse the functional capacity in patients hospitalized for acute exacerbation of COPD. Although there are no studies directly correlating BODE with 6MST, previous studies have shown a significant correlation with other tests to assess functional capacity in individuals with COPD, such as the 6MWT and step test. As demonstrated in a previous study (Regueiro et al., 2009), the performance on the 6MWT of individuals with COPD showed a strong negative correlation with BODE. When correlated with the step test, another study demonstrated that there was a weak negative correlation between the performance in this test and the degree of impairment in the BODE index (Pessoa et al., 2014). The results of the present study indicate that the greater the impairment of the impact of COPD on quality of life (CAT), the worse the functional capacity in patients hospitalized for acute exacerbation of COPD. Although there are no studies directly correlating CAT with 6MST, previous studies have shown a significant correlation of the score with other tests for assessing functional capacity in individuals with COPD, such as the 6MWT and the 6-min pegboard and ring test. A previous study (Silva et al., 2013) had already demonstrated a significant negative correlation between CAT and the distance covered in the 6MWT, validating this questionnaire in the population of individuals with COPD. Also, another study (Jones et al., 2012) had already found a weak correlation between CAT and the performance of the 6MWT to assess responsiveness to a pulmonary rehabilitation program. A recent study also assessed functional capacity in hospitalized patients with exacerbated COPD, but in activities with the upper limbs (6-min pegboard and ring test), and demonstrated a moderate correlation of the test with CAT (Felisberto et al., 2018).

About the risk of a floor effect, is also important to note that, based on the percentage of patients in the sample who were able to adequately perform the 6MST (all patients were able to perform the test, with no dropout or the presence of important clinical alterations that required interruption of its execution) and evaluating the strong correlation between the 6MST and the 6MWT, a test that does not have a floor effect in this population, we were able to demonstrate the removal of the floor effect of the 6MWT in patients hospitalized for an acute exacerbation of COPD. This allows the 6MST to be used to assess the functional capacity of patients in the hospital setting, facilitating its use in situations such as the planning of individually designed therapeutic strategies and the optimization of exercise prescription, enabling a follow-up of their physical improvement, as well as the monitoring the evolution of the functionality and clinical condition in hospitalized patients with acute exacerbation of COPD.

Regarding repeatability, in our study, the average number of cycles performed in the second test was greater than the average number of cycles performed in the first test (an approximate difference of 14 cycles), but it was not considered statistically significant (p = 0.0575). Despite not having the minimum clinically important difference of 20 cycles suggested by a previous study (Pichon et al., 2015), a factor that may justify this result is probably the sample number, which was calculated to answer the main objective of the study - the concurrent validity of 6MST concerning the 6MWT to assess functional capacity in individuals with COPD. When calculating the number of sample to answer the specific repeatability objective that presented an 80% power, the estimated necessary number was 49 individuals. Although we are not able to conclude, our results corroborate previous studies (Borel et al., 2010; Coquart et al., 2014), which found the average performance higher in the second 6MST performed compared to the first 6MST. These authors suggest as justifications for this event (average of higher results in the second test), the possible learning effect in the performance of the second test, as well as the possibility of technical peculiarities of the stepper, such as heating the hydraulic shock absorbers of the device, causing less resistance to effort and influencing performance.

The present study has some limitations. Despite having reached the sample size calculation, as described in the statistical analysis, one of the limitations is the fact that this study was carried out in a single center and, therefore, had a

reduced number of individuals composing the sample, which could influence the extrapolation of the findings to other hospitals. Likewise, as described earlier, as we did not calculate the sample specifically for the repeatability analysis, our data on this aspect could not provide a more definitive answer. Another limitation is that we did not assess the patients' inspiratory capacity during the test, minute by minute, so that we could more closely monitor the development of DH.

CONCLUSION

It could be concluded that 6MST is a valid test for evaluating functional capacity in hospitalized patients with exacerbated COPD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital Sirio-Libanes (approval protocol 3.432.823). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: DR and WY. Data curation: DR, AT, and WY. Formal analysis: DR, AT, and WY. Investigation: DR. Methodology: DR, AT, and WY. Project administration: DR and WY. Supervision: WY. Writing—original draft: DR and WY. Writing—review and editing: DR, AT, and WY.

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SYSTEMATIC REVIEW
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Preoperative Physical Activity Level and Exercise Prescription in Adults With Obesity: The Effect on Post-Bariatric Surgery Outcomes

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This systematic review summarizes current evidence on the relation between preoperative physical activity (PA) levels with bariatric surgery (BS) outcomes and on the beneficial role of preoperative exercise/PA program among BS candidates. This systematic review suggests that candidate patients accumulating the preoperative PA level improved several BS outcomes. These improvements were reported mainly for anthropometric and cardiometabolic parameters and physical function. Observed improvements manifested during a distinct period of time in response to a wide variety of exercise programs. Evidence on the preoperative PA level as well as on preoperative exercise implementation on BS outcomes is advocated despite the small number of participants and lack of control. Thus, further studies are required to explore the most effective and suitable form of exercise prescription prior to BS while considering physical and psychological limitations of obese patients.

Keywords: bariatric surgery, physical activity, exercise intervention, pre-operative, health outcomes

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INTRODUCTION

Since severe obesity is associated with several health, physical, and psychological impairments (WHO Consultation on Obesity, 2000; Flegal et al., 2007; Engin, 2017; Pan et al., 2017), bariatric surgery (BS) is widely accepted as a valuable strategy to improve these alterations (Jabbour and Salman, 2021) and related comorbidities (Brethauer et al., 2011; Schauer et al., 2012; Li et al., 2014; Ardestani et al., 2015; Sams et al., 2016) in both short and long term. Despite all of these promising attributes, the large intersubject variabilities in the number of intra- and postoperative complications, as well as the length of operating time and hospital stay, remain unexplained (Fernandez Jr et al., 2004; Steinbrook, 2004; Nguyen et al., 2013).

While the exact reason for this large intersubject variability of BS outcomes is unclear, it seems that a greater preoperative fitness level and an elevated insulin sensitivity are linked to

Abbreviations: BMI, body mass index; BS, bariatric surgery; BW, body weight; C, abdominal circumference; F, female; FFM, fat-free mass; FM, fat mass; GC, group control; GI, group with intervention; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; HR, heart rate; IT, intervention trial; LS, longitudinal study; M, male; OS, observational study; PA, physical activity; PEBQ, physical exercise belief questionnaire; REE, resting energy expenditure; TEE, total energy expenditure; W/H, waist-to-hip ratio; WC, waist circumference; RCT, randomized controlled study; and TFEQ, three-factor eating questionnaire.

TABLE 1 | PICOS criteria for inclusion of studies.

Parameter	Inclusion criteria
Population	Bariatric surgery candidates
Intervention	Preoperative physical activity and/or exercise intervention
Comparator	Preoperative vs. postoperative
Outcomes	Fitness level, body weight and composition, physical activity level, physical functioning, and muscular performance, aerobic fitness, metabolic parameters, and hospital stay
Study design	Randomized control trial, intervention trial, and prospective studies

better post-BS outcomes (Gilbertson et al., 2017), while a lower cardiorespiratory fitness (i.e., VO2max < 15.8 ml/kg/min) is associated with a longer operating time, intubation duration, estimated blood loss during surgery, and more frequent cardiovascular complications (Gilbertson et al., 2020).

In their pilot trial, Gilberston et al. (2020) reported that prescribing aerobic exercise at the preoperative stage in addition to standard medical care induced significant improvements in postoperative BS outcomes when compared to standard medical care alone. According to these authors, these improvements may be mediated by fitness-related adaptations, including a reduction in adipose tissue-derived hormones, preservation of lean mass, and enhanced metabolic flexibility. Additional studies are still necessary to better profile the potential benefit of adding aerobic exercise and/or other forms of exercise to improve health parameters in bariatric patients. Thus, enhancing fitness indicators and metabolic parameters, prior to surgery, may improve patient outcomes (McCullough et al., 2006; Gilbertson et al., 2017).

The purpose of the present systematic review was to review the available evidence for the beneficial health impact of adding exercise to SC preoperatively and to address metabolic health and surgical outcomes compared to SC alone in patients receiving BS. Moreover, this systematic review highlights the exercise form and modality being implemented in obese patients.

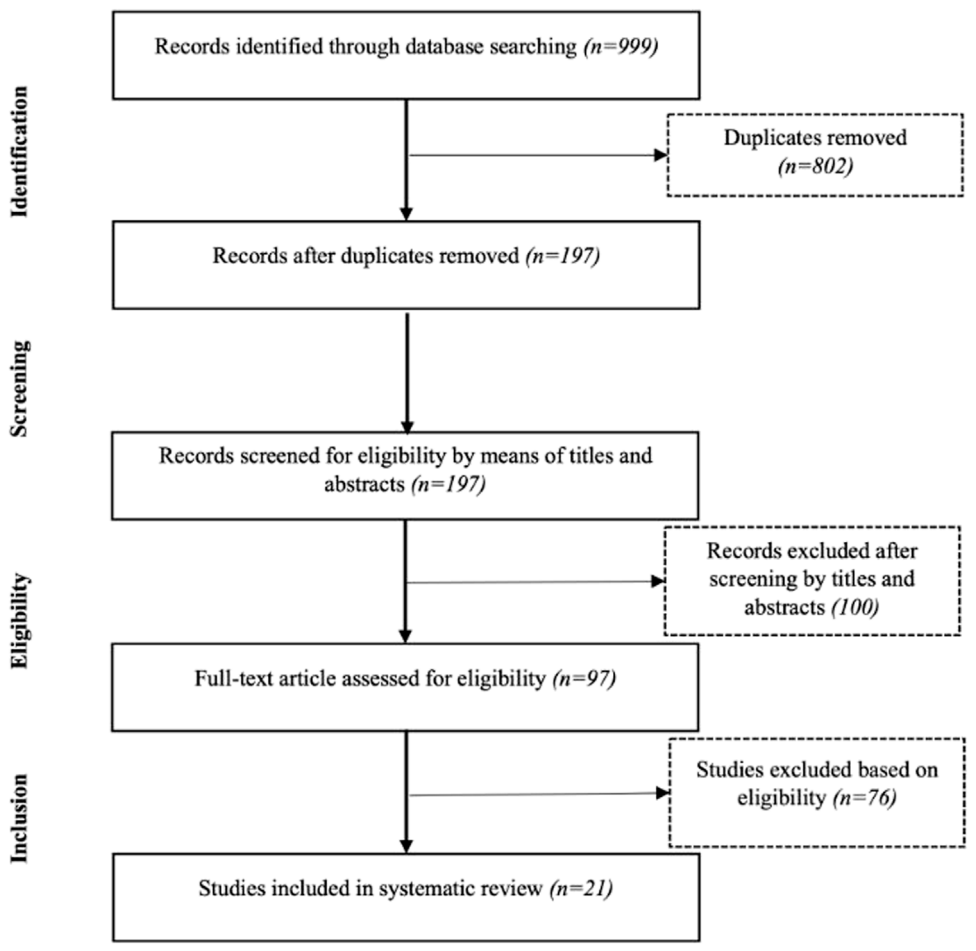

FIGURE 1 | Flow diagram of included and excluded studies included in this systematic review using the recommendations in the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.

TABLE 2 | Baseline characteristics of studies included in the review.

Author (year)	Study design	Population		Baseline BMI (SD)	Type of intervention	Type of bariatric surgery	Assessment methods	Assessment period	Main outcomes
		Age in years; mean (SD) or median [25–75 percentile]	Gender	BMI in kg/m^2; mean (SD) or median [25–75 percentile]					
Baillot et al. (2013)	RCT	40.8 [37.6–47.5]	Eight F and four M	51.4 [43.8–53.1]	Endurance and strength training	Not specified	Bioimpedance scale, symptom-limited cardiac exercise test, 6-min walk test, sit-to-stand test, half-squat test, and arm curl test	Pre- and 12 weeks post-training	Anthropometric measures and physical fitness
(Baillot et al. (2016)	RCT	43.2 (9.2)	GC (11 F and four M) and GI (12 F and three M)	47.5 (8.1)	Endurance and strength training	Not specified	Bioimpedance scale; symptom-limited exercise test; 6-min walk test; sit-to-stand test; half-squat test; arm curl test; PEBQ; and International PA Questionnaire-Short Form	Pre- and 12 weeks post-training	Anthropometric measures; physical fitness; and physical activity level
Baillot et al. (2017)	RCT	GI [44.8 (39.6–54.7)]; GI2 [45.1 (38.6–55.1)]; and GC [43.5 (37.0–46.2)]	Six F	GI [46.6 (39.2–48.5)]; GI2 [44.4 (40.7–53.5)]; and GC [48.4 (40.6–53.3)]	Endurance and strength training	Not specified	Bioimpedance scale; symptom-limited cardiac exercise test; 6-min walk test; sit-to-stand test; half-squat test; arm curl test; and PEBQ	Pre- and 12 weeks post-training	Anthropometric measures; physical fitness; exercise beliefs; and telehealth perception
Baillot et al. (2018)	RCT	GI [44.5 (8.8)] and GC [41.1 (10.3)]	GI (11 F and two M) and GC (nine F and three M)	Not reported in the text	Endurance and strength training	Roux-en-Y gastric bypass or sleeve gastrectomy	Physical activity intensity and total daily energy expenditure; bioimpedance scale; symptom-limited cardiac exercise test; 6-min walk test; sit-to-stand test, half-squat test; arm curl test; and PEBQ	Pre- and 12 weeks post-intervention and 2 weeks pre- and, 3, 6, 9, and 12 months post-BS	Number of steps; PA intensity; physical fitness; exercise beliefs; and anthropometric measures
Bond et al. (2015b)	RCT	GI [44.2 (9.2)] and GC [48.1 (8.1)]	GI (34 F and six M) and GC (31 F and four M)	GI (45.6 (7.0) and GC (44.4 (5.8))	PA intervention	Not specified	PA (SenseWear Armband) and body mass	Pre- and 6 weeks post-intervention	Daily bout-related moderate-to-vigorous PA and body mass
Bond et al. (2015a)	RCT	GI [44.2 (9.2)] and GC [48.1 (8.1)]	GI (34 F and six M) and GC (31 F and four M)	GI [45.6 (7.0)] and GC [44.4 (5.8)]	PA intervention	Not specified	PA (SenseWear Armband); demographic questionnaire; and body mass	Pre- and 6 weeks post-intervention	Moderate-to-vigorous PA and number of steps per day

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TABLE 2 | (*Continued*) Baseline characteristics of studies included in the review.

Author (year)	Study design	Population		Baseline BMI (SD)	Type of intervention	Type of bariatric surgery	Assessment methods	Assessment period	Main outcomes
		Age in years; mean (SD) or median [25–75 percentile]	Gender	BMI in kg/m^2; mean (SD) or median [25–75 percentile]					
Bond et al. (2017)	RCT	GI [46.4 (9.1)] and GC [47.9 (6.8)]	GI (20 F and two M) and GC (11 F and three M)	GI [46.7 (7.1)] and GC [44.4 (7.1)]	PA intervention	Not specified	PA (SenseWear Armband)	Pre-, post-intervention (6 weeks), and post-BS (6 months)	Moderate-to-vigorous PA and number of steps per day
Daniels et al. (2018)	RCT	44.9 (10.2)	16 F	Not reported	Resistance training	Roux-en-Y gastric bypass surgery	Air displacement plethysmography (BodPod); magnetic resonance imaging; and 1-repetition maximum	Pre- and 12 weeks post-training	Fat-free mass; muscle cross-sectional area; muscular strength; and muscle quality
Funderburk and Callis, (2010)	RCT	GI (37.25) and GC (49.3)	Six F (three in each group)	Not reported	Aquatic exercise	Gastric by-pass surgery	Short-Form Health Survey version 2; Obesity Adjustment Scale; Beck Depression Inventory; specialized weight scale; and 6-min walk test	Pre- and post-training	Psychosocial status; depression; adjustment to obesity; and physical status
Gilbertson et al. (2020)	IT	GI [45.6 (4.8)] and GC [39 (5.3)]	GI (seven F) and GC (six F and one M)	GI [43.9 (4.2)] and GC [46.4 (3.0)]	Aerobic exercise	Roux-en-Y gastric bypass Or sleeve gastrectomy	Matsuda index; indirect calorimetry; VO_2 peak; air displacement plethysmography (BodPod); and mixed meal tolerance test	Pre- and post-training	Insulin sensitivity; metabolic flexibility; aerobic fitness; body composition; and adipokines level
Marcon et al., (2011)	IT	42.5 (12.5)	Seven M and 23 F	48.3 (7.2)	Aerobic exercise	Not specified	Body mass; 6-min walking test; blood pressure; and Framingham score risk	Pre- and post-training	Body mass; BMI; functional capacity; and cardiometabolic risk
Marcon et al. (2017)	RCT	GI [43.4 (2.3)]; GI2 [50.1 (2.8)]; and GC [42.5 (2.7)]	GI (18 F and four M); GI2 (17 F); and GC (16 F and two M)	GI [50.8 (9.6)]; GI2 [45 (4.1)]; and GC [47.1 (7.6)]	Low-intensity exercise program	Not specified	Anthropometric; 6-min walking test; resting heart rate; post-exercise heart rate; pre- and post-exercise respiratory rate; oxygen saturation; and estimated VO_2 peak	Pre- and post-training	Body mass; BMI; functional capacity; and cardiometabolic risk
Alger-Mayer et al. (2008)	LS	45.3 (8.9)	120 F and 30 M	52.2 (9.8)	No intervention	Gastric bypass surgery	Body mass	Pre- and 3 years and 4 years post-BS	Body mass and BMI

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TABLE 2 | (*Continued*) Baseline characteristics of studies included in the review.

Author (year)	Study design	Population		Baseline BMI (SD)	Type of intervention	Type of bariatric surgery	Assessment methods	Assessment period	Main outcomes
		Age in years; mean (SD) or median [25–75 percentile]	Gender	BMI in kg/m^2; mean (SD) or median [25–75 percentile]					
Hickey et al. (1999)	IT	Not found	Not found	Not found	Endurance training	Not specified	Blood test	Not found	Fasting plasma insulin and glucose and lipid concentration
King et al. (2008)	OS	44.6 (11.2)	153 M and 604 F	47.4 (7.6)	No intervention	Not specified	PA (the StepWatch 3 Activity Monitor)	Pre-BS	Steps/day
King et al. (2012)	LS	46 [37–55]	241 F and 69 M	45.4 [41.7–51.2]	No intervention	Not specified	PA (the StepWatch 3 Activity Monitor)	Pre- and 1-year post-BS	Steps/day
Still et al. (2007)	LS	45 Ardestani et al. (2015)	692 F and 192 M	51.3 Li et al. (2014)	Medical, psychological, nutritional, and surgical interventions and education	Roux-en-Y gastric bypass	Anthropometric measures	Pre- and 1-year post-BS	Body mass
Colles et al. (2008)	OS	45.2 (11.5)	103 F and 26 M	44.3 (6.8)	No intervention	Laparoscopic adjustable gastric banding	Anthropometric measures; the Cancer Council Victoria Food Frequency Questionnaire; the Three-Factor Eating Questionnaire; the Beck Depression Inventory; the Physical Component Summary; and the Baecke Physical Activity Questionnaire	Pre- and 4 and 12 months post-BS	Body mass; BMI; total energy expenditure; body composition; TFEQ score; the Beck Depression Inventory score; the Physical Component Summary score; and the Baecke PA scores
Browning et al. (2014)	OS	43.3 Nguyen et al. (2013)	145 F and 27 M	43.8 (5.1)	No intervention	Laparoscopic adjustable gastric banding	Anthropometric measures; presence of comorbidities; and the International Physical Activity Questionnaire (IPAQ)	Pre- and 3, 6, and 12 months post-BS	Body mass; BMI; and PA intensity
Brandenburg and Kotlowski (2005)	IT	46.3 [28–64]	55 F and 15 M	55.3 [36–88]	Behavior modification program	Roux-en-Y gastric bypass	Anthropometric measures and Bariatric surgery questionnaire	1 year post-BS	Body mass; BMI; patient demographics; health information; lifestyle habits; and program information

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TABLE 2 | (*Continued*) Baseline characteristics of studies included in the review.

Author (year)	Study design	Population		Baseline BMI (SD)	Type of intervention	Type of bariatric surgery	Assessment methods	Assessment period	Main outcomes
		Age in years; mean (SD) or median [25–75 percentile]	Gender	BMI in kg/m^2; mean (SD) or median [25–75 percentile]					
García-Delgado et al. (2021)	RCT	40 Ardestani et al. (2015)	14 F and one M	46.7 (5.9)	Control group: therapeutical, educational, and cognitive-behavioral therapy; intervention group: therapeutical, educational, and cognitive-behavioral therapy + hysical conditioning and respiratory muscle training program	Not specified	Anthropometric measures; clinical history, physical examination and basic blood tests; EuroQol-5D–5L questionnaire, MEDAS, Eating Disorder Inventory, and Hospital Anxiety and Depression Scale; 6-min walking test, handgrip strength, pulmonary function, and obstructive sleep apnea	Pre-intervention, post-intervention, and post-BS	Body mass; body composition; comorbidities; changes in eating behaviors; health-related quality of life; functional capacity; length of hospital stay after surgery; and short-term complications of surgery

RCT, randomized controlled study; F, female; M, male; GC, group control; GI, group with intervention, LS, longitudinal study; OS; observational study; TFEQ, the Three Factor Eating Questionnaire; PEBQ, physical exercise belief questionnaire; PA, physical activity; BS, bariatric surgery; IT, intervention trial; BMI, body mass index; MEDAS, Mediterranean Diet Adherence Screener questionnaire.

MATERIALS AND METHODS

Eligibility Criteria

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The Population, Intervention, Comparator, Outcomes, and Study design (PICOS) approach was used to identify the inclusion criteria (**Table 1**). Studies that have examined the effects of implementing a structured exercise or a physical activity intervention before BS on postoperative outcomes "body composition, weight loss, physical activity level, performance, and metabolic parameters" were eligible for inclusion. The studies were included in the current systematic review if they met the following criteria: 1) published in peer-reviewed journals, 2) included adult participants, and 3) compared BS outcomes pre- and/or postoperatively. The studies were excluded if they 1) reported only subjective measures, 2) were not written in English, or 3) were retrospective. Moreover, review articles were not included in the current systematic review.

Literature Search Strategy

Literature searches were conducted in four electronic databases, including PubMed, Institute for Scientific Information (ISI) Web of Knowledge, Web of Science, and SPORTDiscus, to identify studies of preoperative exercise intervention or preoperative PA practices using the search terms "bariatric surgery" or "weight loss surgery" or "obesity surgery" or "weight reduction surgery" or "biliopancreatic diversion" or "laparoscopic band" or "lap and" or "gastric band" or "gastric bypass" or "gastroplasty" or "gastric sleeve" or "sleeve gastrectomy" and "preoperative exercise intervention" or "preoperative physical activity" or "preoperative lifestyle modification."

The search was completed with a manual search of reference lists of key articles. Since the scope of this review is large in terms of outcome measures, a systematic review and not a meta-analysis was performed.

Study Selection

The final screening was performed by the principal investigator (GJ) based on the relevance of the inclusion and exclusion criteria and the identified items for assessing the effects of preoperative exercise intervention on anthropometric characteristics and body composition (e.g., body mass, body fat, and BMI), physical performances (e.g., muscular strength and physical capacity), cardiorespiratory fitness and function (e.g., oxygen uptake and heart rate), energy expenditure and metabolism parameters (e.g., resting metabolic rate, insulin resistance, and lipid profile), and hospital stay in obese adults of both genders undergoing BS using PICOS criteria. If the citation showed any potential relevance, the abstract was screened. When abstracts indicated potential inclusion, full-text articles were reviewed.

TABLE 3 | Pre- vs. postoperative body composition, weight loss, physical activity level, performance, and metabolic parameters.

Author (year)	Exercise/PA intervention type	Supervised	Standard medical care and/or outpatient control period	Intervention period	Outcome	Pre-post	Control group
Baillot et al. (2013)	30 min of endurance activity (treadmill and walking circuit) and 20–30 min of strength exercises (upper body, lower body, and trunk)	Yes	Yes	12 weeks	1- body weight (kg)	*	
					2- BMI (kg/m2)	*	
					3- FFM (kg)	*	
					4- FM (kg)	*	
					5- 6MWT (m)	*	
					6- arm curl test (n)	*	
					7- sit-to-stand test (n)	-	
					8- half-squat test (s)	-	
					9- QOL	*	
Baillot et al. (2016)	30 min of endurance activity (treadmill and walking circuit) and 20–30 min of strength exercises (upper body, lower body, and trunk)	Yes	Individual lifestyle counseling intervention	12 weeks	1- body weight (kg)	-	-
					2- fat mass (%)	-	-
					3- SBP (mm Hg)	-	-
					4- DBP (mm Hg)	-	-
					5- 6MWT (m)	-	*
					6- 6MWT perceived exertion	-	-
					7- 6MWT pain (% of subjects)	*	*
					8- 6MWT heart cost	-	*
					9- 6MWT pain intensity scores	*	-
					10- sit-to-stand test (n)	-	-
					11- half-squat test (s)	-	*
					12- arm curl test (n)	-	*
					13- vigorous PA (min/week)	*	*
Baillot et al. (2017)	In-home TelePreSET [supervised twice weekly using videoconferencing] endurance and strength training	Yes	Yes	12 weeks	1- 6MWT distance (m)	*	*
					2- heart cost (m/beats min−1)	*	*
					3- sit-to-stand repetition (n)	*	*
					4- half-squat test time (s)	*	*
					5- arm curl repetition (n)	*	*
					6- maximal aerobic capacity (METS)	*	*
Baillot et al. (2018)	30 min of endurance activity (treadmill and walking circuit) and 20–30 min of strength exercises (upper body, lower body, and trunk)	Yes	Individual lifestyle counseling intervention	12 weeks	1- 1-Y after BS BMI	*	
					2- 1-Y after BS steps (n)	*	
					3- 1-Y after BS light PA (h/day)	*	
					4- 1-Y after BS moderate PA (h/day)	*	
					5- 1-Y after BS 6MWT heart cost	*	
					6- 1-Y after BS half-squat test	*	
Bond et al. (2015b)	Individual face-to-face counseling sessions walking	No	Not reported	6 weeks	1- MVPA (minutes/day)	*	*

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TABLE 3 | (*Continued*) Pre- vs. postoperative body composition, weight loss, physical activity level, performance, and metabolic parameters.

Author (year)	Exercise/PA intervention type	Supervised	Standard medical care and/or outpatient control period	Intervention period	Outcome	Pre-post	Control group
	exercise performed at a moderate intensity and in bouts ≥10 min by 30 min/day. A secondary goal was to increase steps taken by 5,000/day				2- physical function	-	*
					3- role-physical	*	*
					4- bodily pain	*	*
					5- general health	-	*
					6- mental health	-	-
					7- vitality	-	*
					8- physical component summary	*	*
Bond et al. (2017)	Individual face-to-face counseling sessions walking exercise performed at a moderate intensity and in bouts ≥10 min by 30 min/day. A secondary goal was to increase steps taken by 5,000/day	Yes	Not reported	6 weeks	1–6-month after BS steps per day (n)	*	*
					2–6-months after BS MVPA (minutes/day)	-	-
Daniels et al. (2018)	Period 1, three training sessions per week (8–10 exercises, 1 set per exercise, at a range of 10–15 repetitions per set and an intensity of 50–60% of one-repetition maximum (1-RM); period 2, weeks 2–7, consisted of progressively higher volume workouts (i.e., 8–10 exercises, 3–4 sets, and 10–15 repetitions), and progressively higher resistance/intensity (70–80% 1-RM); and period 3 consisted of the remaining 5 weeks of the 12-week resistance-training program. To increase the resistance/intensity (>80% 1-RM) of the exercises from period 2 and decreasing the number of repetitions to 8–12	Yes		12 weeks	Body weight (kg)	*	-
					Stands for leg press (kg)	*	*
					Stands for leg extension (kg)	*	*
					Stands for quadriceps cross-sectional area (cm^2)	-	-
					Stands for whole thigh cross-sectional area (cm^2)	-	-
					Stands for muscle quality leg press	*	*
					Stands for muscle quality leg extension	*	*
Funderburk and Callis (2010)	60 min of aquatic exercises including endurance and strength exercises	Yes	Not reported	12 weeks	1- body weight (kg)	*	-
					2- SBP(mm Hg)	*	-
					3- DBP (mm Hg)	*	-
					4- 6MWT (m)	*	-
					5-RPE	*	-
					5- QOL	*	-
					6- depression score	-	-
					7- physical functioning	*	*
					8- role-physical	*	*
					9- general health	*	*
					10-vitality	*	*
					11- bodily pain	-	-
					12- social functioning	-	-
					13- role-emotional	-	-
					14- mental health	-	-
Gilbertson et al. (2020)	Home basis walking at 65–85% of the HR peak for 30 min per day and 5 days per week	No	Met with dieticians, attended an education session and were cleared for bariatric	30 days	1- body weight (kg)	-	-
					2- BMI (kg/m^2)	-	-
					3- FFM (kg)	*	*

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TABLE 3 | (*Continued*) Pre- vs. postoperative body composition, weight loss, physical activity level, performance, and metabolic parameters.

Author (year)	Exercise/PA intervention type	Supervised	Standard medical care and/or outpatient control period	Intervention period	Outcome	Pre-post	Control group
			surgery by a psychologist + for 2 weeks prior to surgery, patients instructed by registered dieticians to consume a meal replacement shake		4-VO2 peak (ml/kg/min) 5- glucose (mg/dl) 6- FFA (mEq/l) 7- insulin (μU/ml) 8- adiponectin 9- resting metabolic rate (kcal/kgBW/d) 8- length of hospital (min)	* - - - * * *	* - - - * - *
Hickey et al. (1999)	Endurance training at 60% of the VO2 peak and each session was 60 min	Yes	A 3-day outpatient control period was used to monitor adequate caloric intake using dietary intake questionnaires	7 days	1- fasting plasma insulin (pmol) 2- body weight (kg) 3- % FM 4- glucose (mg/dl) 5- lipid (mg/dl) 6- VO2 peak	* - - - - -	
Marcon et al. (2011)	Low intensity endurance training; one session per week, consisting of 209 min of exercise and 10 min stretching	Yes	Not reported	24 weeks	1- body weight (kg) 2- BMI (kg/m^2) 3- SBP (mm Hg) 4- DBP (mm Hg) 5- TC (mg/dl) 6- HDL-C (mg/dl) 7- LDL-C (mg/dl) 8- TG (mg/dl) 9- glucose (mg/dl) 10- 6MWT (m)	* * * * * * * * * *	
Marcon et al. (2017)	Aerobic and stretching exercises performed in two weekly sessions of up to 25 min each, and patients were encouraged to increase the number of steps walked daily for 4 months	Yes	Routine treatment for the control group and support group sessions for lifestyle modification (for EXER + CBT group)	4 months	1- weight (kg) 2- BMI (kg/m2) 3-heart rate exercise (bpm) 4- SBPrest (mmHg) 5- SBP post-exer (mmHg) 6- DBPrest (mmHg) 7- DBP post-exer (mmHg) 8- HDL-C (mg/dl) 9- TC (mg/dl) 10- TG (mg/dl) 11- glucose (mg/dl)	* * * * * * * * * * *	* * * * * * * * * * *
García-Delgado et al. (2021)	15–20 min of physical conditioning consisting of four resistance exercises with elastic bands (2–3 sets of 15 repetitions per exercise) and 10 min of respiratory muscle training consisting of incentive spirometry, respiratory exercises, and inspiratory muscle training.	Yes	Standard medical care for the control group. In addition, the intervention group performs a specific prehabilitation program	16 weeks	1- body mass 2- body composition 3- comorbidities 4- eating behaviors 5- health-related quality of life 6- functional capacity 7- hospital stay post-surgery 8- short-term complications of surgery	Small sample size no. statistics	Small sample size no. statistics

6MWT, 6-min walking test; n, number; SBP, systolic blood pressure; DBP, diastolic blood pressure; VO2 peak, maximum oxygen consumption; TC, total cholesterol; TG, triglycerides; FPG, fasting plasma glucose; FPI, fasting plasma. insulin. (, 0.05; **, 0.01).*

TABLE 4 | Preoperative physical activity and its effects on candidates' outcomes.

Authors (year)	Physical activity form	Physical activity measure	Evaluation period	Outcome	Result
Alger-Mayer et al. (2008)	Patients given exercise "advice"	None	Pre-BS	Body mass	↓ Pre-BS body mass
Bond et al. (2015b)	Helping patients to adopt behavior change	The SenseWear Armband monitor (SWA; BodyMedia, Inc., Pittsburgh, PA)	Pre and post-BS	Daily moderate-to-vigorous physical activity; daily steps; and body mass	↑ Pre- and post-BS; ↑ pre and post-BS; and ↓ post-BS in comparison to the control group
Colles et al. (2008)	Standard advice regarding recommended postoperative eating behaviors and exercise patterns	Validated self-report and Questionnaire Physical Component Summary score of the Medical Outcomes Trust Short Form-36 (SF-36)	Pre and post-BS	Body mass and Beck Depression Inventory-depression score	↓ 4 and 12 months post-BS and ↓ 12 months post-BS
King et al. (2008)	Habitual PA	Objective evaluation of total PA and peak PA intensity; [StepWatch™ 3 Activity monitor (SAM, OrthoCare Innovations, Washington, D.C.)]	Pre-BS	BMI	↓ BMI
King et al. (2012)	Not reported	The StepWatch™ 3 Activity monitor; mean step/day, active minutes/day, and high-cadence minutes/week	1 year post-BS	PA level	Although gains in PA may be smaller among patients with higher preoperative PA, preoperative PA had the strongest positive association with post-operative PA.
Still et al. (2007)	Patients encouraged to use pedometer and walk "8,000 steps/day"	Not reported	Pre- and 12 months post-BS	Body mass	↓ in comparison to control group; ↓ 12 months post-BS

BS, bariatric surgery; PA, physical activity; BMI, body mass index.

RESULTS

Study Selection and Description

Our primary research identified 999 records, including 802 duplicates (**Figure 1**). After screening titles, abstracts, and full texts, 21 studies were included in our final analysis, and the characteristics of these studies are displayed in **Table 2**.

Out of 21 studies, 19 were prospective cohorts (Hickey et al., 1999; Still et al., 2007; Alger-Mayer et al., 2008; Colles et al., 2008; Funderburk and Callis, 2010; Marcon et al., 2011; King et al., 2012; Baillot et al., 2013; Browning et al., 2014; Bond et al., 2015a; Bond et al., 2015b; Baillot et al., 2016; Baillot et al., 2017; Bond et al., 2017; Marcon et al., 2017; Baillot et al., 2018; Daniels et al., 2018; Gilbertson et al., 2020; García-Delgado et al., 2021) and compared pre- to post-BS or pre- to postintervention outcomes in adult patients (**Table 2**). In total, 14 studies introduced an intervention pre-BS, of which 10 were randomized controlled trials that used a structured exercise program (Funderburk and Callis, 2010; Marcon et al., 2011; Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Marcon et al., 2017; Baillot et al., 2018; Daniels et al., 2018; Gilbertson et al., 2020; García-Delgado et al., 2021), three studies used a physical activity program (Bond et al., 2015a; Bond et al., 2015b; Bond et al., 2017), and one study used a lifestyle modification program (Still et al., 2007) (**Table 2**). Among the 14 studies with an intervention, 11 studies performed a pre- to postintervention comparison, nine with exercise (Funderburk and Callis, 2010; Marcon et al., 2011; Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Marcon et al., 2017; Daniels et al., 2018; Gilbertson et al., 2020), and two with PA intervention (Bond et al., 2015a; Bond et al., 2015b), while the remaining three studies performed a pre- to post-BS comparison (Still et al., 2007; Bond et al., 2017; Baillot et al., 2018) (**Table 2**).

Pre- vs. Post-Training Body Composition Changes and Weight Loss

Nine studies reported the effect of exercise training on anthropometric variables, among which five studies found no changes induced by the intervention (**Table 3**). Baillot et al. (2013) found a reduction in body mass, BMI, and fat mass after 12 weeks of supervised combined endurance and strength training (PreSET). In another study, Baillot et al. (2018) compared BMI between usual care and PreSET groups on seven different occasions (preintervention, 12 weeks postintervention, 2 weeks pre-BS, and 3, 6, 9, and 12 months post-BS). They found that the PreSET group experienced a greater decrease in BMI than the usual care group at 9 and 12 months post-BS. Furthermore, (Marcon et al. (2011 and 2017) reported larger decreases in body mass and BMI in the experimental groups than in the control group in two studies (**Table 3**). Funderburk and Callis (2010) reported a reduction in body mass after 12 weeks of supervised aquatic exercises, without a difference between the aquatic exercise and control groups. The rest of the studies did not find any anthropometric differences when comparing pre- to postintervention states or when

comparing experimental (with exercise intervention) to control groups (without exercise intervention) (Hickey et al., 1999; Baillot et al., 2016; Baillot et al., 2017; Daniels et al., 2018; Gilbertson et al., 2020) (**Table 3**).

Pre- vs. Post-Training Effects on Physical Fitness Parameters

Nine studies reported the effect of exercise intervention on physical fitness parameters (Funderburk and Callis, 2010; Marcon et al., 2011; Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Marcon et al., 2017; Baillot et al., 2018; Daniels et al., 2018; Gilbertson et al., 2020), of which two found no changes in measured parameters induced by the intervention (Marcon et al., 2017; Gilbertson et al., 2020) (**Table 3**). All four studies that used concurrent training (endurance and strength training) reported improvement in cardiovascular and/or muscular fitness parameters (Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Baillot et al., 2018). Baillot et al. (2013 and 2016) compared patients' baseline measures to 12 weeks post-training. They found an improvement in the 6-min walk test (6MWT) distance, percentage of theoretical 6MWT distance reached, 6MWT heart cost, half-squat test, and arm curl test. No differences were found in the sit-to-stand test or maximum aerobic capacity. In another study, Baillot et al. (2017) performed within (pre- and 12 weeks post-training) and between groups (training vs. conventional care group) comparisons. Compared to baseline measures, 6MWT distance, sit-to-stand repetitions, arm curl repetitions, and maximal aerobic capacity improved after 12 weeks of training. However, only the 6MWT distance, arm curl repetitions, and 6MWT heart cost improved in the training group compared to the conventional care group (**Table 3**). Another study between-group comparison revealed an improvement in 6MWT heart cost and the half-squat test for the training compared to the conventional care group (Baillot et al., 2018). Notably, the 6MWT distance was found to improve in two studies after aerobic training programs (Marcon et al., 2011) and aquatic exercise programs (Funderburk and Callis, 2010). Finally, only one study evaluated the effect of a 12-week resistance training program and found improvements in leg press strength, leg extension strength, and leg press muscle quality (Daniels et al., 2018) (**Table 3**).

Pre- vs. Postintervention Effects on Postoperative Complications and Hospital Length Stay

Fourteen studies introduced an intervention, among which only four studies reported health-related parameters (Marcon et al., 2011; Baillot et al., 2016; Marcon et al., 2017; Gilbertson et al., 2020) (**Table 3**). Baillot et al. (2016) reported that BS candidates who were committed to a 12-week exercise intervention were protected from worsening of musculoskeletal pain. Gilbertson et al. (2020) tested the effect of a pre-BS aerobic exercise intervention on insulin sensitivity, metabolic flexibility, adipokines, and length of hospital stay. Marcon et al. (2011) evaluated the effect of an aerobic exercise program on the cardiometabolic risk of BS candidates. A significant decrease in systolic and diastolic pressure and the Framingham risk score was found after 6 months of the supervised aerobic exercise program (**Table 3**).

DISCUSSION

In general, patients awaiting BS have a reduced physical fitness level and impairments in several metabolic variables and body composition before surgery. Although BS results in significant weight loss and body composition changes after surgery, it remains uncertain whether other health outcomes (e.g., fitness, metabolic, and cardiorespiratory parameters) are sufficiently improved and how long the improvements can be maintained. The present systematic review highlights the importance of implementing PA and/or exercise interventions close to the candidate's date of surgery (Bond et al., 2006; Bond et al., 2015a; Bond et al., 2015b; Bond et al., 2017). Such interventions could procure many health benefits during the preoperative period (e.g., improved fitness level and PA levels) and in postoperative outcomes (e.g., reduced BS-related complications and reduced hospital length of stay) among BS candidates. Therefore, a preoperative PA/exercise intervention could be an ideal approach to maximize the BS benefits and to offer a successful transition toward improving postoperative lifestyle behaviors among BS candidates. Nevertheless, studies with larger cohorts are needed to confirm these results, and a longer follow-up period (>1 year) is required to understand more fully the impact of a preoperative intervention on postoperative outcomes.

Pre- vs. Post-Training Body Composition Changes and Weight Loss

Six studies examined the effect of PA and/or exercise intervention on body composition parameters pre- vs. postoperatively (Brandenburg and Kotlowski, 2005; Still et al., 2007; Alger-Mayer et al., 2008; Colles et al., 2008; Browning et al., 2014; Baillot et al., 2018), and five studies reported relevant data regarding body composition parameters before and after PA/exercise intervention in preoperative BS candidates (Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Marcon et al., 2017; Baillot et al., 2018) (**Table 4**). Some studies showed a significant decrease in preoperative body mass (Still et al., 2007; Alger-Mayer et al., 2008; Bond et al., 2015a) or BMI (King et al., 2008) after an intervention. For these studies, the positive impact procured by a PA intervention prior to BS may be explained primarily by the improvement in PA and physical fitness levels among BS candidates, which is an important step toward improving their overall health parameters. Despite these promising results, it remains difficult to attribute all of these improvements solely to PA considering that many limitations have not been addressed, such as the lack of any control of PA (in the majority of cases, patients were only advised to practice PA) without excluding the

interference of BS candidates' existing conditions (such as BMI and comorbidities) as well as their diet and lifestyle prior to surgery.

Nonetheless, the effect of PA and exercise interventions on postoperative anthropometric parameters has been considered an interesting topic. Studies using interdisciplinary individual lifestyle counseling and helping BS patients adopt behavioral changes (e.g., to be active) (Still et al., 2007; Colles et al., 2008; Bond et al., 2015b) reported significant decreases in body mass during the post-BS period (**Table 4**). However, the lack of a control group, the small sample size, and the specificity of the sample may limit the generalizability of these results.

Other studies (Funderburk and Callis, 2010; Baillot et al., 2016; Baillot et al., 2017; Daniels et al., 2018; Gilbertson et al., 2020) applying supervised exercise training during the preoperative period found no significant pre- to postoperative changes in any body composition parameters between the intervention and usual care groups. It seems that BS induces a strong influence on weight loss and therefore can mask any eventual effect of preoperative intervention. In a study by Baillot et al. (2016), participants received lifestyle counseling for an average of 10.4 ± 4.0 months (5.8 ± 1.8 dietician and 5.6 ± 1.8 PA specialist visits) before inclusion in the study. Thus, significant changes might have occurred before inclusion in the surgical treatment option. In contrast, Baillot et al. (2018) reported a larger BMI decrease in the BS group undergoing preoperative exercise intervention (PreSET) compared to that in the usual care group and attributed this improvement to the higher loss of fat-free mass (FFM) in the PreSET group. Moreover, Gilbertson et al. (2020) reported a significant decrease in FFM in participants undergoing preoperative home-based walking for 30 min per day. In addition, Marcon et al. (2017) reported similar results after 4 months of aerobic and stretching exercises. These discrepancies among results might be primarily attributed to the duration and form of intervention. Moreover, the characteristics of patients prior to the PA and exercise intervention must be considered. In fact, many BS candidates encounter remarkable difficulties (e.g., musculoskeletal problems, preoperative fitness level) that might affect their exercise tolerance and adherence, consequently limiting or reducing the PA/exercise intervention benefits. Therefore, more support in selecting an appropriate activity along with a feasible monitoring technique is highly required in such a context.

Pre- vs. Post-Training Effects on Physical Fitness Parameters

Reduced physical fitness, reported mostly in BS candidates, may affect the BS results. Current evidence supporting the importance of increasing the physical fitness level in BS candidates is not abundant but is promising. In fact, several studies found that a preoperative intervention based on exercise or Pa that aimed at improving physical fitness and performance indicators (e.g., strength, 6MWT distance, and maximal aerobic capacity) among individuals awaiting BS may be an effective strategy to improve the BS candidates' overall health parameters and their BS outcomes (Funderburk and Callis, 2010; Baillot et al., 2013; Baillot et al., 2016; Baillot et al., 2017; Marcon et al., 2017; Baillot et al., 2018; Daniels et al., 2018). In this regard, Baillot et al. (2013 and 2016), in their randomized controlled trial, reported a significant increase in some physical function parameters assessed with a test battery (6MWT, sit-to-stand, half-squat, and arm curl test) after 12 weeks of supervised exercise training either with or without an individual lifestyle counseling intervention. One study by Gilbertson et al. (2020) reported significant increases in the VO_2 peak among BS candidates after adding preoperative aerobic exercise to standard medical care. To the best of our knowledge, this is the only study to investigate the effect of a preoperative PA intervention on aerobic performance. The increase in the VO_2 peak may be associated with a shorter operation time and length of hospital stay and with prevention of muscle loss along with a concomitant increase in the PA level among BS candidates. However, the mechanism underlying these improvements remains to be studied.

The PA level is an interesting parameter that has been evaluated. In a long-term study (1 year after surgery), Baillot et al. (2018) reported that the addition of preoperative supervised exercise training to individual lifestyle counseling improved PA levels and submaximal physical fitness 1 year post-BS. Similarly, King et al. (2012) reported a gain in the PA level 1 year after surgery among patients with higher preoperative PA levels. Other short-term studies (Bond et al., 2015a; Baillot et al., 2016; Bond et al., 2017) reported similar results regarding PA levels in response to preoperative intervention. A small amount of evidence suggests that improvements in PA in response to a preoperative PA/exercise intervention may mainly be attributed to improvements in PA barriers, social interactions, and feelings of embarrassment (Bond et al., 2015a; Baillot et al., 2016). In addition, the impact of the overall improvement in the fitness level following a PA/exercise intervention aimed at improving the physical fitness of preoperative BS candidates cannot be overlooked. However, the small sample size and exclusion criteria applied in the aforementioned studies prevent their generalization to all subjects awaiting BS. Moreover, the study recruitment process was limited to volunteers who were able to frequently visit the facility and were without major functional limitations.

Pre- vs. Postintervention Effects on Health Parameters, Postoperative Complications, and Length of Hospital Stay

Only four studies have explored a limited number of health parameters (Marcon et al., 2011; Baillot et al., 2016; Marcon et al., 2017; Gilbertson et al., 2020). Baillot et al. (2016) looked at the effect of a 12-week exercise program on musculoskeletal pain and found that BS candidates can be protected from worsening of pain associated with daily life activities. Additional studies are required to confirm the impact of PA on musculoskeletal pain before and after BS. In a study by

Gilbertson et al. (2020), patients undergoing preoperative EX + SC prior to bariatric surgery had a shorter length of hospital stay than patients undergoing preoperative SC. Two studies by Marcon et al. (2011 and 2017) reported a significant decrease in systolic and diastolic blood pressure and the Framingham risk score after 6 months of a supervised aerobic exercise program.

To date, the mechanisms responsible for such improvement have not been explored, although current evidence clearly favors a preoperative PA/exercise intervention for facilitating better postoperative outcomes. Few studies have investigated the role of preoperative interventions on BS outcomes. The differences in study design and the lack of randomized controlled trials decrease the evidence level of the results. Moreover, the heterogeneity of activities performed (with or without supervision), the inclusion criteria, and the interference of covariates (e.g., participant characteristics) were not well controlled. Finally, it is important to mention that the included studies were very small and had a short follow-up time, thus making the results less convincing. The data provided by this review did not consider BS procedures (i.e., type of surgery), making the results difficult to interpret.

In conclusion, this review summarizes the benefits of a preoperative PA/exercise intervention among BS candidates and highlights the importance of such strategies as a component of medical therapy. A good understanding of the beneficial effect of improving the preoperative physical condition on postoperative outcomes is highly recommended for future interventional studies to potentiate the beneficial effect of BS among obese candidates. Based on significant evidence, including optimized weight loss post BS, reduced cardiovascular risk, and increases in patients' regular PA and fitness levels, there is a need to implement a PA/exercise program before BS to promote and optimize BS outcomes. Nevertheless, there is a need for future investigations in this field to determine the most appropriate form(s) of PA/exercise intervention according to the patient profile.

AUTHOR CONTRIBUTIONS

GJ and RI were involved in the conceptualization of the study, data analysis, and the writing of the manuscript. NB was involved in data assessment, data analysis, and the writing of the manuscript. All authors approved the final version of the manuscript.

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Muscle strength, aerobic capacity, and exercise tolerance are impaired in left ventricular assist devices recipients: A pilot study

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Background: Left ventricular assist devices (LVAD) are increasingly being used as a therapy for advanced heart failure, both as a bridge to heart transplant and, given the rapid advances in the LVAD's functionality and safety, and constant lack in availability of donor organs, as long-term destination therapy. With the diffusion of such therapy, it is crucial to assess patients' muscle strength, aerobic capacity and exercise tolerance, to improve their functional capacity.

Methods: 38 LVAD recipients (33 men and five women) were included. Exercise testing including a maximal cardiopulmonary exercise test (CPET), handgrip, isometric and isokinetic strength testing of knee and ankle flexion/extension, and Romberg balance test in three conditions (eyes open, eyes closed, double task). Given the small and heterogeneous final sample size, a mostly descriptive statistical approach was chosen.

Results: 12 participants were classified as "Obese" (BMI>29.9). The most common comorbidities were type II diabetes and chronic kidney disease. Only 12 participants were able to successfully complete all the assessments. CPET and isokinetic strength trials were the least tolerated tests, and the handgrip test the best tolerated. Mean VO_2 peak was 12.38 ± 3.43 ml/kg/min, with 15 participants below 50% of predicted VO_2 max, of which 6 below 30% VO_2max. Mean handgrip strength was 30.05 ± 10.61 Kg; 25 participants were below the 25° percentile of their population's normative reference values for handgrip strength, 10 of which were below the 5° percentile. Issues with the management of the external pack of the LVAD and its influence on the test limited the validity of the balance tests data, therefore, no solid conclusions could be drawn from them. VO_2 peak did not correlate with handgrip strength or with any of the lower limb strength measures.

Conclusion: LVAD recipients show greatly reduced functional capacity and tolerance to exercise and exercise testing, with low overall strength levels. As strength variables appear to be independent from VO_2 peak, different lower limbs strength tests should be explored to find a tolerable alternative in this population, which is subjected to muscle wasting due to old age, reduced tissue perfusion, side effects from the pharmacological therapies, and prolonged periods of bedrest.

KEYWORDS

left ventricular assist device, exercise test, muscle strength, exercise tolerance, aerobic capacity, postural control

Introduction

Heart failure is a significant cause of morbidity and mortality, affecting at least 64 million patients in the world (Lippi and Sanchis-Gomar, 2020). Left ventricular assist devices (LVAD) are increasingly being used as a therapeutic option for advanced heart failure, both as a bridge to heart transplant (HT) and, given the rapid advances in the LVAD's functionality and durability, and the limited availability of donor organs, as long-term destination therapy (Kirklin et al., 2017). The implant of a LVAD comes with a 48% reduction in mortality from any cause (Rose et al., 2009) and an improvement in quality of life (Rogers et al., 2010), although the patient often faces difficulties transitioning into their new life, and can suffer from a number of adverse events, like bleedings and infections (Adams and Wrightson, 2018; Han et al., 2018).

There is an increase in the number of LVAD candidates, caused by individuals with advanced and end-stage heart failure who do not have access to (or are not eligible for) HT. Consequently, there is an increasing number of LVAD recipients (more than 3,000 new implants in 2019 alone, according to the Society of Thoracic Surgeons' 2020 annual report; Molina et al., 2021), who are living longer with the implant. Following these perspectives, it is crucial to assess the patients to determine their level of functional capacity, in order to improve the patient's physical conditioning. In this optic, while aerobic-cardiovascular variables (e.g., power, capacity, etc.) is abundantly monitored and assessed in those patients, muscular strength-related conditioning is less well expressed in scientific literature.

Noteworthy, handgrip strength has been found to be inversely correlated with hospital length of stay after LVAD implantation (Yost and Bhat, 2017), and sarcopenia diagnosed before the implantation is associated with a decreased 6-months survival ratio (Roehrich et al., 2022). To our knowledge, the only study investigating the lower limbs' strength performance of LVAD recipients was conducted by Kerrigan et al. (2013), who reported data only about isokinetic test of knee extension, concluding that peak torque was strongly associated with patient-reported health status. Previous research has found significant correlation between handgrip strength and VO_2 peak both in younger (Dag et al., 2021; Ajepe et al., 2022) and older people (Sugie et al., 2018); however, no such relationship is apparent between leg strength and VO_2 peak, at least in older, inactive individuals (Matthews et al., 2020).

On one hand, strength quantification is fundamental in predicting the independence of daily living activities in LVAD patients, in fact muscular strength has also been found to be a determinant of physical disability (Savage et al., 2011); on the other hand, understanding muscular strength in LVAD recipients is relevant to increase the overall fitness through structured training protocols, in fact resistance training can provide an increase in muscle strength, aerobic power and capacity and quality of life in this population (Giuliano et al., 2017), compared with usual care (Ganga et al., 2017). However, the programs used are highly variable, as no official guidelines have been published at this point (Alonso et al., 2021). In the light of these viewpoints and in the lack of a solid body of knowledge, this study aims to analyze strength parameters in subjects with LVAD, and the potential correlation between muscular strength and aerobic capacity in LVAD patients.

Materials and methods

Between 2015 and 2019, 38 LVAD recipients (33 men and five women, age: 58.1 ± 7.64 years) received a medical examination. All participants gave their informed consent, and the study was approved by the local ethics committee (Padova University).

Participants' height and weight (body mass) were measured respectively with a stadiometer (Ayrton Corporation, Model S100, Prior Lake, MN, United States), and an electronic scale (Home Health Care Digital Scale, Model GS 51 XXL, Beuer Gmbh, Ulm, Germany). Height and weight (body mass) were used to calculate body mass index (BMI) of the participants. The medical history, medical examination, and cardiopulmonary exercise test were administered by a physician with Sport Medicine specialization. Exercise capacity was assessed by incremental, ECG-monitored, cardiopulmonary exercise testing (Jaeger- Masterscreen-CPX, Carefusion, Germany). Both tests were randomly performed on treadmill (modified

Bruce protocol) and bicycle (protocol +10 W/min), and performed until exhaustion (Borg rating of perceived exertion (RPE) ≥18/20).

The New York Heart Association (NYHA) classification was applied to classified patients in one of four categories based on their limitations during physical activity. The limitations/symptoms are in regards to normal breathing and varying degrees in shortness of breath and or angina pain (Heart Foundation, 2014). Charlson comorbidity index (Charlson et al., 1987) were used to classify the patient health status in relation to comorbid conditions.

Before muscular strength tests, a warm-up was performed to reduce the risk of injuries. A 60-s recovery period was allowed between all testing procedures. Dominant and non-dominant handgrip strength was measured with a calibrated dynamometer (Baseline, Elmsford, NY, United States). Grip handle was adjusted to accommodate the size and comfort of the participant's hand, and the elbow was flexed to 90° to guarantee the strongest grip strength measurement (Mathiowetz et al., 1985). Three trials for each hand were performed, and the mean of dominant hand was used for percentile identification. Lower limb muscle strength tests were performed with subjects seated on the multi-joint system with the backrest angled at 90° to the seat. Belts were fastened across the thighs, pelvis, and shoulders to minimize body movements and to optimally isolate the movement of the knee and ankle joints. Subjects folded their arms across their chest and were not permitted to hold on to the equipment during the tests. During knee trials, the lever fulcrum was aligned with the rotation axis of knee, with the lateral femoral epicondyle used as the point of reference, and the shin pad was placed 2 cm above the medial malleoli. Instead, during the ankle trials, the lever fulcrum was aligned with the medial malleoli. Before all isokinetic tests, the weight of the legs and the ankles were noted and a gravity adjustment was made using the computer software. During the maximal isometric knee extension, the lever arm was set at 75° extension, calculated from the maximum knee extension of each participant. Subjects had to push as much as possible, with leg, on the shin pad for 5 s. Conversely, during maximal isokinetic knee extension and flexion participants pushed and pulled the shin pad as fast as possible for five times uninterrupted. The velocity of isokinetic movement was set at 90°s. When testing the maximal isometric ankle plantar and dorsal flexion, the lever arm was set at 30° of plantar flexion, calculated from the maximum ankle dorsal flexion (0°) of each participant, and the foot was fixed on a support with two stripes. Subjects had to push down and pull up the ankle support as much as possible for 5 s, during extension and flexion trials. Finally, during maximal isokinetic ankle plantar and dorsal flexion, participants had to push down and pull up the ankle support as fast as possible for five times continuously. The velocity of this isokinetic movement was set at 90°s. All data were acquired at 1,000 Hz, and analyzed as absolute strength, and relative strength (absolute strength/body mass). This protocol was previously used and validated for older adults (Bergamin et al., 2017).

Postural control was measured by means of posturography with an ARGO stabilometric platform (RGMD, Genova, IT) in three conditions: Eyes open, eyes closed and dual task (counting backwards aloud), as previously outlined in Zanotto et al. (2020). Each test was performed with subject upright with feet together and the arms at sides. In front of it, a blackboard was placed to the distance of 3 m. During the Romberg test with eyes open, the subject has to fixed a reference point located on the blackboard for 30 s. During the Romberg test with eyes closed, the subject has to stay on the platform for 30 s with closed eyes. In dual-task condition, participants had to stand as still as possible in Romberg position, with eyes open, counting backwards aloud, starting from a randomly selected number, in steps of one, as fast and as accurately as possible for the entire duration of the test (Yardley et al., 1999; Bergamin et al., 2014). All participants performed randomly the three balance tests three times.

Given the study design, a descriptive statistical approach was chosen (data is presented as mean ± S.D.), and the Pearson product moment correlation coefficients between VO_2 peak and strength measurements were computed ($\alpha = 0.05$). All data was managed using Microsoft Excel 365 (Microsoft Corporation, 2018).

Results

38 participants underwent functional capacity, strength, and balance assessments, as reported in the method section. Results from tests are presented in Table 1, and data are expressed as mean ± standard deviation. Mean BMI was 27.26 ± 3.97, with 12 participants classified as "Obese" (BMI>29.9). The physical and clinical evaluations were conducted after a mean period of 59.19 ± 46.37 weeks. Data on pharmacological therapies revealed the participants were taking, on average, 10 different medications each day. In addition to the medications taken for the management of heart failure, pain and infection risk, it should be noted that 47% of the participants were taking at least one psychotropic medication (antidepressants being the most common).

The most common comorbidity was type II diabetes (7 participants), followed by chronic kidney disease, dyslipidemia and hypertension (5 participants), with a mean Charlson Comorbidity Index (computable for 23 participants) of 3.48 ± 1.41 resulting in a 60% 10-years survival rate (drug therapies and comorbidities are described in Table 2). Participants tested showed a poor overall physical condition and a weak functional capacity, with most of the participants (12 out of 18 participants) who completed the CPET examination falling into the "C" (10 participants) or "D" (2 participants) functional class of the NYHA classification.

TABLE 1 Test Results.

Outcome	*n*	Mean ± S.D.	Outcome	*n*	Mean ± S.D.
VO_2 Peak (L/min kg)	18	12.38 ± 3.43	Isokinetic extension strength, left ankle (kg)	24	12.81 ± 7.18
Handgrip, dominant hand (kg)	38	30.05 ± 10.61	Isokinetic flexion strength, left ankle (kg)	24	16.29 ± 5.72
Handgrip, non dominant hand (kg)	37	26.59 ± 9.73	Romberg test, eyes open, sway path (mm/s)	31	19.44 ± 6.36
Isometric extension strength, right knee (kg)	28	120.86 ± 44.52	Romberg test, eyes open, sway area (mm^2/s)	31	46.47 ± 22.98
Isometric extension strength, left knee (kg)	28	109.57 ± 38.95	Romberg test, eyes open, anterior-posterior oscillations (mm)	31	25.68 ± 8.03
Isokinetic extension strength, right knee (kg)	27	80.68 ± 28.84	Romberg test, eyes open, lateral oscillations (mm)	31	31.95 ± 7.62
Isokinetic flexion strength, right knee (kg)	27	35.47 ± 17.96	Romberg test, eyes closed, sway path (mm/s)	31	30.65 ± 12.86
Isokinetic extension strength, left knee (kg)	27	73.14 ± 21.71	Romberg test, eyes closed, anterior-posterior oscillations (mm)	31	35.32 ± 11.55
Isokinetic flexion strength, left knee (kg)	27	37.02 ± 18.94	Romberg test, dual task, sway path (mm/s)	29	23.02 ± 8.19
Isometric extension strength, right ankle (kg)	24	19.38 ± 10.53	Romberg test, dual task, anterior-posterior oscillations (mm)	29	26.63 ± 9.67
Isometric flexion strength, right ankle (kg)	24	28.51 ± 7.31	Romberg test, eyes closed, sway area (mm^2/s)	31	94.88 ± 63.60
Isometric extension strength, left ankle (kg)	23	17.58 ± 12.30	Romberg test, eyes closed, lateral oscillations (mm)	31	41.77 ± 12.90
Isometric flexion strength, left ankle (kg)	23	26.88 ± 6.51	Romberg test, dual task, sway area (mm^2/s)	29	53.24 ± 28.68
Isokinetic extension strength, right ankle (kg)	25	11.83 ± 6.51	Romberg test, dual task, lateral oscillations (mm)	29	31.80 ± 8.79
Isokinetic flexion strength, right ankle (kg)	25	16.48 ± 6.72			

TABLE 2 Characteristics of Study Participants.

Outcome	Result (n of participants)
Age (years)	58.11 ± 7.64 (36)
Stature (m)	1.70 ± 0.07 (36)
Body mass (kg)	79.26 ± 14.54 (36)
BMI (kg/m^2)	27.26 ± 3.97 (36)
Comorbidities (type)	Type II Diabetes Mellitus (7), hypertension (5), dyslipidemia (5), chronic kidney disease (5), hernia (inguinal, umbilical, hiatal) (3), gastritis (2), Gallbladder calculosis or cholecystectomy (2), other (10)
Comorbidities (*n*. of)	No com. (5), 1 com. (8), 2 com. (4), 3 com. (4), 4 com. (3), 5 com. (1), ≥6 com. (0)
Charlson Comorbidity Index	3.48 ± 1.41 (23)
Drugs (type)	anticoagulant (18), diuretics (18), proton pump inhibitors (18), Antiarrhythmic (13), non-steroidal antinflammatory (12), mineral supplements (Calcium, sodium, magnesium, potassium) (12), iron supplement (13), antihypertensive (10), antithrombotic (9), antibiotic (9), beta-blocker (8), thyroid hormonal drugs (8), statin (8), antidepressive (Other) (5), diabetes medication (4), gout medication (4), benzodiazepine (4), vitamin supplements (B, D) (4), hepatic medication (3), alpha-blocker (3), antipsychotic (3), antidepressive (SSRI) (2), other (10)
Drugs (n. of)	4 drugs (1), 7 drugs (3), 8 drugs (4), 9 drugs (3), 10 drugs (2), 11 drugs (5), 14 drugs (2), 15 drugs (1), 16 drugs (1)

Seven participants had a HeartWare HVAD System implanted, which was recalled in 2021 after a series of malfunctions (Medical Device Recalls, 2021); fortunately, no pump-related adverse events were registered during or between the tests, and no systematic difference in performance could be observed between these and other participants.

Only 12 participants (32%) were able to successfully complete all the assessments. CPET and isokinetic strength trials were the least tolerated tests (completed by 18 and 22 participants, respectively). Handgrip test has been completed bilaterally by 37 participants. Mean VO_2 peak was 12.38 ± 3.43 ml/kg/min, and the mean handgrip strength (dominant hand) was 30.05 ± 10.61 Kg. The balance tests were completed by 31 participants in the eyes open/closed condition, and by 29 participants in the dual task condition; in the eyes open condition mean sway path was 19.44 ± 6.36 mm/s, mean sway area was 46.47 ± 22.98 mm^2/s, mean anterior-posterior oscillations were 25.68 ± 8.03 mm, and mean lateral oscillations were 31.95 ± 7.62 mm. In the eyes closed condition mean sway path was 30.65 ± 12.86 mm/s, mean sway area was 94.88 ± 63.60 mm^2/s, mean anterior-posterior oscillations were 35.32 ± 11.55 mm, and mean lateral oscillations were 41.77 ± 12.90 mm. In the dual task condition, mean sway area was 23.02 ± 8.19 mm/s, mean sway area was 53.24 ± 28.68 mm^2/s, mean anterior-posterior oscillations were 26.63 ± 9.67 mm, and mean lateral oscillations were 31.80 ± 8.79 mm.

TABLE 3 Correlation.

Outcome	*n*	VO_2 peak correlation (Pearson's r)	*p* value
Handgrip, dominant hand (kg)	18	−0.31	0.21
Handgrip, non dominant hand (kg)	18	−0.1	0.71
Isometric extension strength, right knee (kg)	14	−0.38	0.18
Isometric extension strength, left knee (kg)	14	−0.41	0.14
Isokinetic extension strength, right knee (kg)	14	−0.3	0.30
Isokinetic flexion strength, right knee (kg)	14	−0.28	0.34
Isokinetic extension strength, left knee (kg)	14	−0.17	0.57
Isokinetic flexion strength, left knee (kg)	14	−0.27	0.34
Isometric extension strength, right ankle (kg)	14	0.26	0.38
Isometric flexion strength, right ankle (kg)	14	−0.25	0.38
Isometric extension strength, left ankle (kg)	13	0.45	0.12
Isometric flexion strength, left ankle (kg)	13	−0.04	0.91
Isokinetic extension strength, right ankle (kg)	14	−0.02	0.95
Isokinetic flexion strength, right ankle (kg)	14	−0.03	0.93
Isokinetic extension strength, left ankle (kg)	13	−0.02	0.96
Isokinetic flexion strength, left ankle (kg)	13	−0.24	0.43

n: number of participants who completed both the VO2 peak and corresponding strength assessment.

No statistically significant correlations were found between VO_2 peak and handgrip strength ($p > 0.2$) and between VO_2 with any of the lower limb strength measures ($p > 0.1$), as outlined in Table 3.

Discussion

The aim of this paper was to characterize some aspects of physical capacity in patients with LVAD. In particular, the strength profile was analyzed using isometric (including the handgrip test) and isokinetic strength tests. Compared to a normal population in terms of age, body mass and gender; in general, muscle strength of LVAD patients appeared as reduced. With more detail, 25 participants were below the 25° percentile of their population's normative reference values for handgrip strength, furthermore 10 of which were below the 5° percentile. Mean isometric knee extension strength was below the reference values found in literature for a comparable (healthy) population; the reference values used are outlined in Šarabon, Kozinc, and Perman (2021), who employed a similar methodology for determining isometric knee strength. Reference values from a healthy population were chosen because, to our knowledge, there are no reference values for LVAD recipients strength parameters. This drop in both upper and lower-body strength could be explained by a few possible reasons. First off, although more active than patients with heart failure, LVAD recipients demonstrated lower levels of physical activity than healthy subjects (Jakovljevic et al., 2014). This condition, in turn, could explain a reduction in muscle mass and functionality, especially in older adults (Rezuş et al., 2020). Secondly, LVAD recipients (and before, heart failure patients) often experienced prolonged and repeated periods of bed rest, which can lead to muscle atrophy (Ikezoe et al., 2011) with potential consequential impairment in terms of muscle strength. Finally, subjects with reduced heart functionality often suffered from skeletal muscle dysfunction, contributing to exercise intolerance (Haykowsky et al., 2011; Bekfani et al., 2020). This vicious circle could resemble positive feedback where, however, muscular strength could progressively decrease over time.

Literature was clear about how upper and lower limb strength contribute to older adults' ability to complete activities of daily living (Wang et al., 2020); knee and ankle extension strength can be used as predictors of loss of autonomy (Buckinx et al., 2019), therefore understanding the decline in strength measure become important for LVAD patients to counteract the risk for disability. Additionally, establishing robust strength training protocols to maintain sufficient muscle function assumes a critical role for these patients.

Analyzing the aerobic-cardiovascular component, as could be expected, aerobic capacity was also compromised in this population: out of the 18 participants who completed the CPET, 15 were below 50% of their predicted maximal VO_2, six of which were below 30% VO_2 max. VO_2 has been previously found to be a predictor of long-term survival in the general population (Ross et al., 2016), and in heart-failure patients (Hsich et al., 2007).

While the association between muscle mass and aerobic capacity waned with aging (Kim et al., 2016), muscle loss appeared to play a pivotal role in age-related decline of VO_2

max (Fleg and Lakatta, 1988). In trained older men and women, instead, the reduction in max O_2 delivery seemed to be the main driving factor of this phenomenon (Proctor and Joyner, 1997), and lower-limb strength was positively correlated with maximal aerobic performance in trained older individuals (Matthews et al., 2020). In LVAD recipients, instead, muscle strength (as measured by isometric and isokinetic tests) was not significantly correlated with VO_2 peak. This could signify that aerobic capacity of those patients was probably so compromised that cardiac performance remained the limiting factor during CPET, which highlighted the importance of performing specific strength tests to accurately describe the functional status of this population.

Another important issue concerned balance test is that all parameters (sway path, sway area, anterior-posterior and medio-lateral oscillations) were greater in closed eyes condition respect to open eyes condition or dual-task condition. This finding suggests that LVAD recipients seem to have a compromised postural control with visual deprivation. This probably due to physical decondition and in some cases for the side effects of certain drug therapies (e.g. antiarrhythmic) or other comorbidities (e.g. diabetes) that may alter proprioception. One aspect to consider is the presence of the LVAD's external battery pack, which may have altered the test. Indeed, this can weigh up to 2 kg with probable alteration in the subject's sway parameters. For these reasons to assess the risk of falls, a graded test, like the Berg balance scale (Berg et al., 1992) could be more appropriate in this population. Alternatively, the risk of falls could be inferred from the knee and ankle peak torque and rate of force development (Bento et al., 2010; Valenzuela et al., 2020), even though more population-specific trials would be needed to confirm this assumption.

The overrepresentation of male participants in this study (33/38 participants) is in line with other reports, where women were less likely to receive a LVAD, since they appear to have poorer outcomes and more frequent adverse events (Joyce et al., 2009; Magnussen et al., 2018; Dayanand et al., 2021). The high prevalence of obesity (over 30% of participants) in the sample should spark action towards weight management strategies in this population. Obesity is a known general risk factor for cardiovascular diseases, and has been found to increase the incidence of infections, neurological complications, and thrombosis in LVAD recipients, affecting short-term survival (Zhigalov et al., 2020). The high percentage of participants under a psychotropic medication therapy is compatible with the psychological challenges faced by these persons in the transitional period following the LVAD implant (Okam et al., 2020).

This study presented several limitations. The first issue is obviously linked to the great difficulty in finding this type of patient and especially in testing them. Secondarily, there was a considerable amount of data loss, mainly related to the health status of the participants. These missing data were not random but came mostly from the participants who could not complete the physical tests (or for whom the tests had to be ended prematurely), which means that these results were computed using only the data from the fittest participants, and thus the means are likely to be overestimated, especially for the outcomes with the lowest sample sizes. Finally, during the recruiting process, we did not impose inclusion/exclusion criteria on the time period between LVAD implant and examination, unavoidably reducing the homogeneity of the sample. This variability is probably a feature of the population itself, which nevertheless makes the interpretation of the data more complex, even compared with the simple normal population in terms of comparison.

In conclusion, LVAD recipients show greatly reduced functional capacity and tolerance to exercise and exercise testing, with low overall strength levels. This complex frame therefore entails a reduced physical function. As strength performance appears to be independent from VO2 peak, strength tests, such as the handgrip strength test, should be included in the physical function assessment; however, given that isokinetic tests, especially of the ankle joint muscles, were poorly tolerated, a different alternative should be explored in this population. Future works on LVAD recipients could focus on comparing different physical testing protocols to find the most suitable compromise between validity and applicability for these persons. Further studies could help establishing reference values for strength and cardiovascular outcomes that are specific to LVAD recipients, to use as starting point when preparing an exercise training protocol and exercise guidelines for LVAD recipients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the University of Padova Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SG and MB designed the work; AG and VB acquired the data; FF and AB analyze the data; LC, AB, and DB provide their intellectual contribution about data interpretation; FF, SG, AE, and MB participate in manuscript writing, give an important

contribution in revising it and the final draft. All authors give their final approval of the version to be published.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Agreement and reliability of lower limb muscle architecture measurements using a portable ultrasound device

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High end ultrasonography devices lack in portability and are expensive. We investigated the agreement and reliability of a handheld and portable ultrasound system for human lower limb muscle architecture measurements. We captured ultrasound images of the rectus femoris (RF), vastus lateralis (VL) and gastrocnemius medialis (GM) in 36 active healthy participants (15 female, 21 male) at 50% of muscle length using the handheld Lumify (L12-4, linear-array 37 mm, Philips Healthcare, Amsterdam, Netherlands) and a high-end laboratory device (ACUSON Juniper, linear-array 54 mm, 12L3, SIEMENS Healthineers, Erlangen, Germany). We compared measurements of muscle fascicle length, pennation angle and thickness. To assess inter-session reliability of the Lumify system, participants were measured twice within 1 week. Comparing RF architecture measurements of both devices resulted in intra-class correlations (ICCs) ranging from 0.46–0.82 and standardized mean difference (SMDs) ranging from −0.45–0.05. For VL, ICCs ranged from 0.60–0.89 and SMDs ranged from −0.11–0.13. ICCs and SMDs for the GM ranged from 0.82–0.86 and −0.07–0.07. Calculating inter-session reliability for RF resulted in ICCs ranging from 0.44–0.76 and SMDs ranging from −0.38–0.15. For VL, ICCs and SMDs ranged from 0.57–0.75 and −0.13–0.02. ICCs for GM ranged from 0.75–0.92 and SMDs ranged from −0.15–0.16. Measurement of muscle thickness demonstrated the highest agreement (ICC ≥0.82) and reliability (ICC ≥0.75) across all muscles. The Lumify system was comparable to a high-end device and reliable for GM measurements. However, agreement and reliability were lower for the RF and VL. Of all evaluated architectural parameters, muscle thickness exhibited highest agreement and reliability.

KEYWORDS

ultrasound, muscle architecture, lumify, comparability, reliability, lower limbs

1 Introduction

Modern day, high-end ultrasonography devices still are relatively expensive and bound to elevators and flat underground for transportation. Although most devices can be moved between assessment rooms, fast transportation across longer distances as well as availability for immediate assessment in the field is limited. Recently, ultrasonography devices consisting solely of a probe, an app, and/or a mobile display were introduced. Exemplars of these devices are, among others, the Philipps Lumify (Philips Healthcare, Amsterdam, Netherlands), the GE Healthcare Vscan Air (GE Medical Systems, Chicago, United States) or the Sonosite Iviz (FUJIFILM Sonosite, Bothell, United States). The devices usually weigh under 500 g, and thus inherit (nearly) unrestrained portability (Toscano et al., 2020). Furthermore, they are cheaper, with purchase prices usually being less than a quarter of high-end devices.

Overall, ultrasonography has many potential applications, and several investigations already examined the feasibility of using portable probes. Toscano et al. (2020) reported high sensitivity and specificity when investigating basic gynecology pathologies using the Lumify or Iviz probes. Johnson et al. (2022) on the other hand determined the Lumify probe to be accurate for measurements of the optic nerve sheath diameter in simulation models. Moreover, other investigations demonstrated that the Lumify probe can be used for point-of-care ultrasound training (Drake et al., 2021) and plastic surgery (Miller et al., 2018).

Apart from these applications, ultrasonography can be used to assess muscle architecture (Esformes et al., 2002; Narici et al., 2003; Franchi et al., 2018; Sarto et al., 2021). Ultrasonography measurements of muscle architecture are valid and reliable (Blazevich et al., 2006; Bénard et al., 2009; Bolsterlee et al., 2015; Scott et al., 2017; Geremia et al., 2019; Franchi et al., 2020; Nijholt et al., 2020). The architecture of a muscle is defined as arrangement of a muscle's fibers relative to the force generation axis and characterized by fascicle length, pennation angle and thickness (Gans and Bock, 1965; Lieber and Frieden, 2000). Muscle architecture, especially of the lower limb, is not only important to aspects of physical performance (Sarto et al., 2021), but also to clinical outcomes (Onambele et al., 2006; Onambélé et al., 2007; Puthucheary et al., 2013; Stenroth et al., 2015; Paillard, 2017; van Alfen et al., 2018; Narici et al., 2021).

Yet, to the best of our knowledge, the comparability and reliability of portable probes to assess muscle architectural parameters has not been investigated. This however is relevant for both, a sports performance and medical context. Portable probes would allow for cost-effective, on-site athlete or patient screenings of muscle architecture as well as diagnosis of pathologies or injury. Furthermore, portable probes can be easily transported to allow for uncomplicated assessment of athletes belonging to different teams or patients in different hospital units. Moreover, due to lower acquisition cost, portable probes might facilitate a broader usage of ultrasonography to assess muscle architecture. This could lead to a better understanding of the significance of muscle architecture and its relation to performance and clinical outcomes. Therefore, the aim of this investigation was to determine the comparability and reliability of a portable probe (Philips Lumify) for lower limb muscle architecture measurements. For this, we compared the portable probe measurements to those of a high-end device and assessed test-retest reliability of the portable probe.

2 Materials and Methods

We conducted B-mode ultrasonography measurements of lower limb muscle architecture in 36 participants (15 female (age: 25.9 ± 2.7 years, height: 166.7 ± 3.9 cm, body mass: 59.9 ± 5.8 kg, skeletal muscle mass: 25.7 ± 2.8 kg, fat mass: 13.3 ± 2.9 kg), 21 male (age: 31.5 ± 7.0 years, height: 177.7 ± 7.2 cm, body mass: 71.5 ± 8.6 kg, skeletal muscle mass: 34.5 ± 5.0 kg, fat mass: 10.6 ± 4.5 kg)). Participants were required to be older then 18 years, healthy and without injury of the lower limbs within the prior 6 months. We asked the participants to refrain from exercise 24 h prior to the measurements. The study protocol was approved by the local ethics committee (Ethics Committee of North-Western and Central Switzerland, approval number: 2020–02,034) and complied with the Declaration of Helsinki. Participants signed an informed written consent prior to the start of the study after receiving all relevant study information.

To test the agreement of a 37-mm linear array portable probe (L12-4, Philips Healthcare, Amsterdam, Netherlands), we used one high-end ultrasonography device with a 56-mm linear array probe (12L-3, Acuson Juniper, SIEMENS Healthineers, Erlagen, Germany) as the gold standard. We used a Samsung Galaxy Tab S6 (Samsung, Seoul, South Korea) as mobile display in combination with the portable Lumify probe.

Subsequently to collection of anthropometric data, we acquired longitudinal, static ultrasonography images of the m. rectus femoris, m. vastus lateralis and m. gastrocnemius medialis. We selected these muscles as they are the most relevant (and most investigated) to functional and clinical outcomes (Onambele et al., 2006; Onambélé et al., 2007; Puthucheary et al., 2013; Stenroth et al., 2015; Paillard, 2017; van Alfen et al., 2018; Narici et al., 2021; Sarto et al., 2021). Participants rested in a supine position for 5 minutes prior to acquisition of rectus femoris and vastus lateralis images. First, we determined and marked 50% of the distance between the proximal and distal muscle tendon junction. Moreover, we marked the muscle midpoint at this location as the middle of the distance between the medial and lateral muscle border assessed by ultrasonography. Following the placement on the region, we

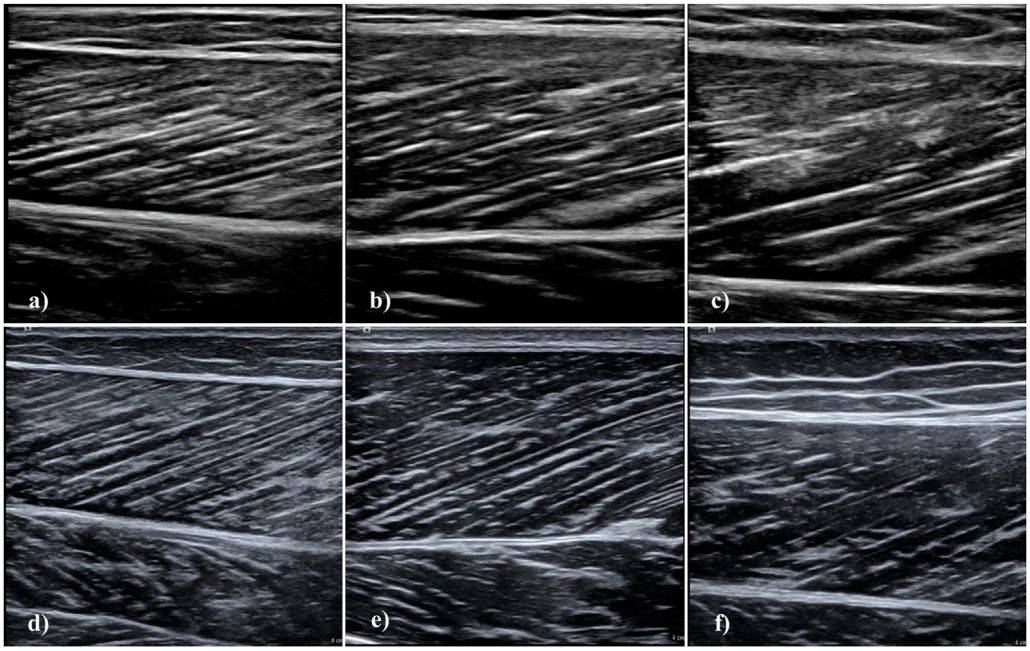

FIGURE 1
Longitudinal muscle ultrasonography images using the Philips Lumify and Siemens Acuson Juniper devices. Images acquired with the Lumify are **(A)** m. gastrocnemius medialis, **(B)** m. vastus lateralis, and **(C)** m. rectus femoris. Images acquired with the Acuson Juniper are **(D)** m. gastrocnemius medialis, **(E)** m. vastus lateralis, and **(F)** m. rectus femoris. Images displayed here were acquired in different participants, except for m. gastrocnemius medialis images.

adapted the orientation of the probe according to fascicle plane (Bolsterlee et al., 2016). Subsequently, we took three images per muscle at this location (Figure 1) (Reeves et al., 2009; Franchi et al., 2015).

For gastrocnemius medialis measurement, participants were transitioned to a dynamometer (Isomed 2000; D. & R. Ferstl GmbH, Hemau, Germany) and placed in a prone position and rested for 5 minutes. The right foot was secured at an angle of 25° plantar flexion and we determined 50% of the distance between the proximal and distal muscle tendon junction (Narici et al., 2003). Again, we assessed and marked medio-lateral muscle midpoint, orientated the probe according to muscle fascicle orientation and acquired three images (Figure 1) (Narici et al., 2003; Bolsterlee et al., 2016). We employed a cross-over design for all muscles, always acquiring images with the high-end device first. We used the same marked locations upon collection of the images with the Lumify probe.

Furthermore, we assessed the test-retest reliability of the Lumify probe. Therefore, participants were invited for a second investigation 1 week later and three images per muscle were collected with the portable probe. Participants reported to the laboratory at the same time of day (± 1 hour). Re-marking of the muscle tendon junctions and muscle midpoints was necessary, as we did not instruct the participants to remark the locations by themselves.

All images were collected by an experienced investigator (PR) with 4 years of experience in acquiring and analyzing ultrasonography images (more than 1,000 acquired images of each of the here investigated muscles). We used a semi-automated tool SMA software (Seynnes and Cronin, 2020) to analyze the images and assess parameters muscle thickness, fascicle length and pennation angle. For each parameter, we computed the mean of at least two images. For every image, SMA computes the dominant fascicle orientation (Seynnes and Cronin, 2020). Then, fascicle length and pennation angle are calculated using the computed orientation and the two detected aponeuroses (Seynnes and Cronin, 2020). Muscle thickness is calculated as the mean distance between the two detected aponeuroses (Seynnes and Cronin, 2020). We used the maximum orientation value in each parameter and one value is calculated for each parameter for further analysis (Seynnes and Cronin, 2020). Analysis results were visually inspected and the analysis parameters were adapted in case the result was erroneous. It was demonstrated SMA is comparable to manual analysis (current gold standard for muscle architecture analysis) in the assessment of muscle thickness, fascicle length and pennation angle (Seynnes and Cronin, 2020).

TABLE 1 Comparability statistics of the portable Lumify system and the high-end device.

Parameter	Mean value Lumify	Mean value high-end	ICC	SEM	SEM%	Mean Bias	SMD
GM FL	55.0 ± 11.2	55.1 ± 11.0	0.84 (0.71,0.92)	4.5 (3.0,5.7)	7.8	0.1 (−12.4,12.5)	0.1 (−0.4,0.5)
GM PA	21.7 ± 5.5	21.4 ± 4.6	0.87 (0.75,0.93)	1.9 (1.4,2.3)	8.4	−0.3 (-5.5,4.9)	−0.1 (−0.5,0.4)
GM MT	18.6 ± 3.5	18.9 ± 3.4	0.89 (0.79,0.94)	1.2 (0.9,1.4)	6.6	0.3 (-3.0,3.5)	0.1 (-0.4,0.6)
RF FL	87.5 ± 13.5	82.7 ± 10.9	0.46 (0.15,0.69)	8.6 (6.7,10.3)	10.7	−4.8 (−28.7,19.1)	−0.5 (−0.9,0.1)
RF PA	15.2 ± 2.7	16.4 ± 2.8	0.42 (0.11,0.66)	1.9 (1.5,2.4)	12.5	1.2 (−4.4,6.7)	0.4 (−0.1,0.9)
RF MT	21.2 ± 3.4	21.4 ± 3.2	0.82 (0.67,0.91)	1.4 (1.1,1.7)	6.5	0.2 (-3.8,4.1)	0.1 (-0.4,0.5)
VL FL	81.2 ± 9.9	79.9 ± 12.0	0.80 (0.64,0.90)	4.9 (3.4,6.1)	6	−1.4 (−14.8,12.1)	−0.1 (−0.6,0.4)
VL PA	14.3 ± 2.8	14.6 ± 2.4	0.60 (0.33,0.78)	1.6 (1.2,2.0)	12.6	0.3 (−4.2,4.8)	0.1 (-0.4,0.6)
VL MT	21.2 ± 2.5	21.1 ± 2.7	0.89 (0.79,0.94)	0.9 (0.6,1.1)	4.3	−0.1 (−2.5,2.4)	−0.1 (−0.5,0.5)

All values calculated for m. gastrocnemius medialis (GM), m. rectus femoris (RF) and m. vastus lateralis (VL) comparing the Lumify probe to a high-end device. Parameters of comparison were fascicle length in mm (FL), pennation angle in ° (PA) and muscle thickness in mm (MT). Mean values ± standard deviation. Consecutive pairwise intra-class correlation coefficient (ICC) and standard error of measurement (SEM) with 95% compatibility interval. Standard error of measurement is also displayed in percent (SEM%). Mean bias and standardized mean bias (SMD) with limits of agreement set to ± 1.96 standard deviations.

2.1 Statistics

All statistical analyses were performed using R software (R Core Team, 2020) (rstudioapi, BlandAltmanLeh, readxl, irr, MBESS packages). Normality was assessed for all parameters using visual inspection (scatterplots, histograms and QQ-plots) and Shapiro-Wilk tests. We compared the portable probe measurement results to the high-end device measurement results for all muscles. For this purpose, we calculated consecutive-pairwise intra-class correlations (ICC), standard errors of measurement (SEM) and percentage standard errors of measurement (SEM%) with 95% compatibility intervals (CI). We classified the ICC values according to Koo and Li (2016) with ICC values of less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.9 are poor, moderate, good and excellent (Koo and Li, 2016). We used Bland–Altman analysis (Bland and Altman, 1986) to test the measurement agreement of the portable probe with the high-end device. We set the limits of agreement to ± 1.96 standard deviations (SD). We calculated the standardized mean bias according to Hopkins et al. (2009), with 0.1, 0.3, 0.6, 1.0 and 2.0 being small, moderate, large, very large and extremely large errors. Furthermore, we investigated test-retest reliability of the portable probe by calculating ICCs, SEMs, SEM% with 95% CI. We classified the ICC values according to Koo and Li (2016) with ICC values of less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.9 indicating poor, moderate, good and excellent reliability (Koo and Li, 2016). We calculated mean bias and standardized mean bias between test sessions. We categorized standardized mean bias as 0.1, 0.3, 0.6, 1.0 and 2.0 for extremely high, very high, high, moderate, and low reliability (Hopkins et al., 2009).

3 Results

Mean values, ICCs, SEMs, SEM%, mean bias, and standardized mean bias of all muscles comparing the portable probe to the high-end device are shown in Table 1. Overall, the gastrocnemius medialis displayed the highest agreement between the two device types and rectus femoris the lowest. Measurement errors of the portable probe for gastrocnemius medialis were small in all parameters. In contrast, measurement errors of the portable probe for vastus lateralis were small for muscle thickness, whereas errors for fascicle length and pennation angle were moderate. Measurement of the portable probe errors for rectus femoris were large for fascicle length and pennation angle, yet muscle thickness measurement errors were small. Considering ICC values, portable probe assessment of architectural parameters in the gastrocnemius medialis according to Koo and Li (2016) ranged from moderate to excellent (95% CIs included). For the rectus femoris and vastus lateralis, ICC values can be classified as low to excellent (95% CIs included), demonstrating a large variability between parameters. Taking the investigated muscles together, ICCs, SEM%, and standardized mean bias for fascicle length ranged from 0.457 to 0.899, 6–10.7%, and −0.45 to 0.01. For pennation angle, ICCs, SEM%, and standardized mean bias ranged from 0.423 to 0.865, 8.4–12.6%, and −0.07 to 0.43. Comparison of muscle thickness resulted in ICCs, SEM%, and standardized mean bias ranges of 0.821–0.889, 4.4–6.6%, and −0.02 to 0.07. Results of the Bland-Altman analysis can be found in Figure 2. There is no indication of heteroscedasticity in all muscles and parameters. Rectus femoris fascicle length seems to be slightly underestimated by the Lumify probe, whereas rectus femoris pennation angle seems to be slightly overestimated (Figure 2).

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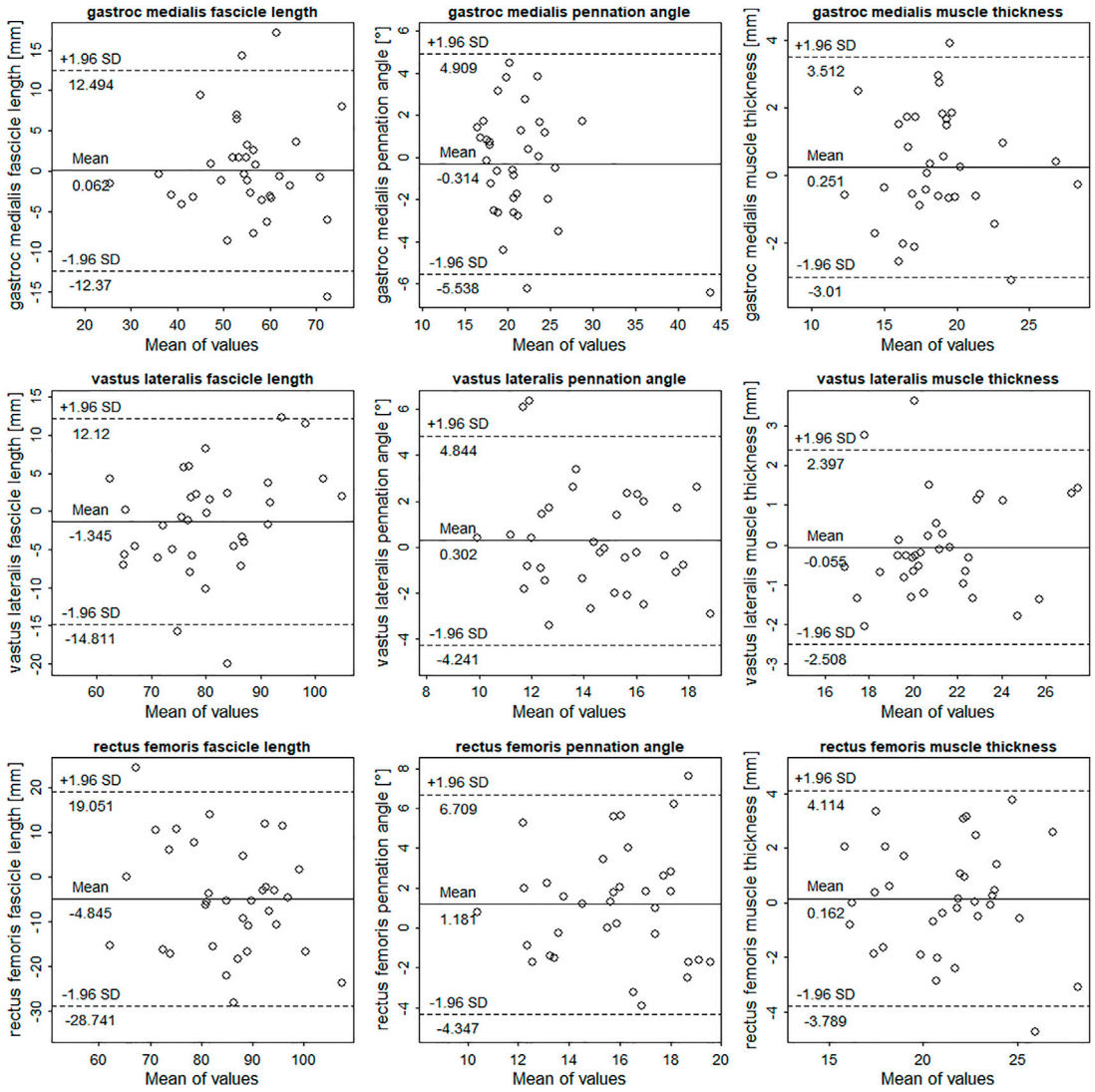

FIGURE 2
Bland-Altman plots comparing the Philips Lumify measurements to measurements of the Siemens Acuson Juniper (here taken as gold-standard). The differences between measurements are plotted against measurement means. Dotted and solid lines illustrate 95% limits of agreement and bias.

Mean values, test-retest ICCs, SEMS, SEM%, mean bias, and standardized mean bias of all muscles for the portable probe are shown in Table 2. According to Hopkins et al. (2009), reliability of the portable probe to assess gastrocnemius medialis muscle thickness, fascicle length, and pennation angle, was extremely high to very high. For vastus lateralis architectural parameters, reliability of the portable probe was extremely high to very high. The portable probe demonstrated very high to high reliability for rectus femoris architecture assessment. Nonetheless, considering ICC values, reliability of the portable probe to assess architectural parameters in the gastrocnemius medialis according to Koo and Li (2016) ranged from moderate to excellent (95% CIs included). For the rectus femoris and vastus lateralis, reliability ranged from low to excellent (95% CIs included), demonstrating a large variability between parameters. Taking the investigated muscles together, ICCs, SEM%, and standardized mean bias for fascicle length ranged from 0.439 to 0.789, 7–11.8%, and −0.15 to 0.15. For pennation angle, ICCs, SEM%, and standardized mean bias ranged from 0.520 to 0.746, 9.4–12.7%, and -0.38 to 0.16. Comparison of muscle thickness resulted in ICCs, SEM%, and standardized mean bias ranges of 0.746–0.921, 5.7–7.8%, and −0.19 to −0.02.

TABLE 2 Test-retest statistics for the Lumify probe.

Parameter	Mean MTP 1	Mean MTP 2	ICC	SEM	SEM%	Mean Bias	SMD
GM FL	55.0 ± 11.2	55.6 ± 8.8	0.79 (0.62,0.89)	4.6 (3.2,5.7)	8.5	−1.6 (−3.9,0.6)	−0.2 (−0.6,0.3)
GM PA	21.7 ± 5.5	20.8 ± 4.4	0.75 (0.55,0.86)	2.5 (1.4,3.6)	9.3	0.9 (−0.3,2.1)	0.2 (-0.3,0.6)
GM MT	18.6 ± 3.5	18.6 ± 3.1	0.92 (0.85,0.96)	0.9 (0.7,1.2)	5.7	−0.1 (−0.5,0.4)	−0.1 (−0.5,0.5)
RF FL	87.5 ± 13.5	85.6 ± 15.1	0.44 (0.13,0.67)	10.8 (7.6,13.3)	11.8	1.9 (−3.4,7.3)	0.2 (−0.3,0.6)
RF PA	15.2 ± 2.7	16.2 ± 3.1	0.52 (0.23,0.73)	1.9 (1.5,2.3)	11.9	−1.0 (−2.0,−0.1)	−0.4 (−0.9,0.1)
RF MT	21.2 ± 3.4	21.9 ± 3.4	0.76 (0.57,0.87)	1.6 (1.2,2.1)	7.8	−0.6 (−1.5,0.2)	−0.2 (−0.7,0.3)
VL FL	81.2 ± 9.9	81.1 ± 11.4	0.72 (0.51,0.85)	5.7 (4.2,7.0)	7.0	0.2 (−2.7,3.0)	0.1 (−0.5,0.5)
VL PA	14.3 ± 2.8	14.7 ± 2.4	0.57 (0.3,0.76)	1.7 (1.3,2.0)	12.7	−0.4 (−1.2,0.5)	−0.1 (−0.6,0.4)
VL MT	21.2 ± 2.5	21.3 ± 2.9	0.75 (0.55,0.86)	1.4 (1.0,1.7)	6.9	−0.1 (−0.8,0.6)	−0.1 (−0.5,0.4)

All values calculated for m. gastrocnemius medialis (GM), m. rectus femoris (RF) and m. vastus lateralis (VL). Parameters of comparison were fascicle length in mm (FL), pennation angle in ° (PA) and muscle thickness in mm (MT). Measurement timepoint (MTP) mean values ± standard deviation. Consecutive pairwise intra-class correlation coefficient (ICC) and standard error of measurement (SEM) and mean bias with 95% compatibility interval. Standard error of measurement is also displayed in percent (SEM%). Standardized mean bias (SMD) with limits of agreement set to ± 1.96 standard deviations.

4 Discussion

Our results indicate that the portable Lumify probe demonstrated small to moderate measurement errors compared to the high-end device with low to excellent ICC values. Test-retest reliability considering standardized mean bias was extremely high to high and ICC values were low to excellent. Whereas agreement and reliability were highest for the measurement of muscle thickness across all muscles, pennation angle measurement in gastrocnemius medialis and vastus lateralis and fascicle length measurement in rectus femoris presented the lowest agreement and reliability. Muscle specific agreement and reliability were highest for gastrocnemius medialis.

Although several studies investigated the test-rested reliability and validity of ultrasonographic measurement of muscle architecture (Blazevich et al., 2006; Bénard et al., 2009; Bolsterlee et al., 2015; Scott et al., 2017; Geremia et al., 2019; Franchi et al., 2020; Nijholt et al., 2020), few tested the agreement of different probes (Cho et al., 2018; Nijholt et al., 2020). Nijholt et al. (2020) compared the measurements of a linear and curved array probe and tested the validity of lower limb muscle thickness, cross-sectional area and echo intensity against MRI. They reported both, the linear and curved array probe, to be reliable (ICC between 0.87 and 0.97) and valid (mean difference between 0.2 and 2.1 cm^2). Cho et al. (2018) investigated the inter- and intra-rater reliability of a dual-probe ultrasonography system and compared the results to a standard ultrasonography device. They reported excellent reliability and agreement for gastrocnemius medialis pennation angle (ICCs >0.9 and SEMs <1°). In contrast to our results, the agreement and validation values reported in these studies indicate higher agreement between the investigated systems. Moreover, reported agreement in validation studies of ultrasonography based muscle architecture assessment using cadaveric dissection was higher as well (Bénard et al., 2009; Kellis et al., 2009). In terms of test-retest reliability of ultrasonographic architecture assessment in all here investigated muscles, ICCs between 0.7 and 0.98, 0.7 to 0.98 and 0.74 to 0.99 have been reported for muscle thickness, fascicle length, and pennation angle, respectively (Narici et al., 2003; Blazevich et al., 2006; Ema et al., 2013; Bolsterlee et al., 2015; Trezise et al., 2016; Geremia et al., 2019; Nijholt et al., 2020; Betz et al., 2021; May et al., 2021; Hagoort et al., 2022). Reported SEM% were between 0.6 and 4.8%, 2–18.9%, and 4.3–23% for muscle thickness, fascicle length, and pennation angle, respectively (Narici et al., 2003; Bénard et al., 2009; Ema et al., 2013; Bolsterlee et al., 2015; Trezise et al., 2016; Oranchuk et al., 2020; May et al., 2021; Hagoort et al., 2022). Compared to the existing literature, solely our gastrocnemius medialis test-rest reliability ICC values for are within the ranges demonstrated in the literature (0.75–0.92 vs. 0.7–0.99). Our test-retest reliability ICC values for rectus femoris and vastus lateralis architecture were lower compared to reported values in the literature (0.44–0.76 vs. 0.7–0.99). Except for muscle thickness measurements, all our test-retest reliability SEM% values are within the ranges demonstrated in the literature. Lower test-retest reliability of the Lumify probe might be explained by inferior image quality compared to high-end devices. Usually, reduced image quality inherits lower pixel contrast and increased signal noise, leading to less visible tissue structures. Because the SMA tool employs spatial filters to detect muscle architectural parameters and aponeuroses, lower image quality thus leads to less accurate detection and therefore less accurate image evaluation.

Apart from the cost-effectiveness and portability advantages of portable probes, there are also some restraints. The extended-field-of-view modality (Weng et al., 1997) is usually not

embedded in portable probes. In combination with the generally reduced transducer width, this limits the applicability of portable probes as fascicle length extrapolation is generally necessary which might result in measurement errors (Franchi et al., 2020). Moreover, assessment of muscle anatomical cross-sectional area in larger muscles (such as vastus lateralis, gastrocnemius medialis or rectus femoris) is therefore impossible using portable probes, except when single images are stitched together. In addition, the processing capabilities of portable probes are limited compared to high end devices, leading to less sophisticated signal post processing. Moreover, less parameters to adapt during image acquisition are available, limiting the options to ideally configure the imaging settings.

The here presented investigation has several limitations. First, we did not use cadaveric dissection as a gold standard to validate the portable probe measurements against, but a high-end device. However, using cadaveric dissection is expensive and access limited. Moreover it was previously demonstrated that ultrasonography is comparable to muscle architectural measurements in cadaveric samples (Bénard et al., 2009; Kellis et al., 2009). We did not use the extended-field-of-view ultrasonography modality to acquire longitudinal muscle images. Even though this might have led to increased accuracy (Franchi et al., 2018; Franchi et al., 2020), this modality is not embedded in the Lumify probe and the analysis software used is not specificized on analyzing extended-field-of-view images (Seynnes and Cronin, 2020). Because we used a fixed scanning order (high-end device first), longer resting period prior to measurements with the Lumify device might have resulted in fluid shifts that could have influenced our results. Nonetheless, no consistent bias is visible in our data. Furthermore, because we did not instruct participants to remark the scanning location of the first session, re-marking could have resulted in slightly altered scanning locations. This might have influenced the test-retest reliability of the Lumify device. Lastly, we only included healthy active adults in our investigation. This might limit the generalizability of our results to other populations.

5 Conclusion

To sum up, the portable Lumify probe demonstrated reliable muscle architecture measurements of lower limb muscles that are comparable to those of a high-end device. Highest reliability and agreement were observed for m. gastrocnemius measurements, lowest for m. rectus femoris. Nonetheless, measurement errors should be considered when interpreting observed longitudinal changes in muscle architecture assessed with the Lumify system. Future investigations should consider including different participant populations, comparing the reliability of different portable systems for muscle architecture assessment as well as including different muscle groups.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

Ethics Statement

The studies involving human participants were reviewed and approved by Ethikkommission Nordwest- und Zentralschweiz (EKNZ). The patients/participants provided their written informed consent to participate in this study.

Author contributions

PR, OF, and MF designed the study. PR and RS collected the ultrasound images. PR and RS performed image and data analysis. PR, OF, and MF wrote the first draft of the manuscript. All authors read and revised the manuscript draft and approved the final manuscript. All authors had access to all the data in the study and PR, OF, and MF verified the data and had final responsibility for the decision to submit for publication.

Conflict of Interest

PR and OF were under contractual agreement with Philips Healthcare. However, Philips Healthcare was neither involved in the design and conduct of the study, nor the analysis. Further, Philips Healthcare did not have any decision making or approval in the submission of this manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Biceps femoris long head morphology in youth competitive alpine skiers is associated with age, biological maturation and traumatic lower extremity injuries

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Lower extremity injuries are common in competitive alpine skiers, and the knee and lower leg are often affected. The hamstring muscles, especially the biceps femoris long head (BFlh), can stabilize the knee and the hip and may counteract various adverse loading patterns during typical mechanisms leading to severe lower extremity injuries. The aim of the present study was to describe BFlh morphology in youth competitive alpine skiers in relation to sex, age and biological maturation and to investigate its association with the occurrence of traumatic lower extremity injuries in the upcoming season. 95 youth skiers underwent anthropometric measurements, maturity offset estimations and ultrasound assessment, followed by 12-months prospective injury surveillance. Unpaired t tests showed that the two sexes did not differ in BFlh morphology, including fascicle length (Lf), pennation angle (PA), muscle thickness (MT) and average anatomical cross-sectional area ($ACSA_{avg}$). In contrast, U16 skiers had longer fascicles than U15 skiers (9.5 $\pm$ 1.3 cm vs 8.9 $\pm$ 1.3 cm, $p < 0.05$). Linear regression analyses revealed that maturity offset was associated with Lf ($R^2 = 0.129$, $p < 0.001$), MT ($R^2 = 0.244$, $p < 0.001$) and $ACSA_{avg}$ ($R^2 = 0.065$, $p = 0.007$). No association was found between maturity offset and PA ($p = 0.524$). According to a binary logistic regression analysis, $ACSA_{avg}$ was significantly associated with the occurrence of traumatic lower extremity injuries (Chi-square = 4.627, $p = 0.031$, $R^2_{Nagelkerke} = 0.064$, Cohen f = 0.07). The present study showed that BFlh morphology is age- and biological maturation-dependent and that BFlh $ACSA_{avg}$ can be considered a relevant modifiable variable associated with lower extremity injuries in youth competitive alpine skiers.

KEYWORDS

muscle morphology, hamstrings, ultrasound imaging, injury prevention, alpine ski racing, youth athletes

Introduction

Competitive alpine skiing is a sport with a high risk of traumatic injuries (Jordan et al., 2017; Spörri et al., 2017). The lower extremities, especially the knee and the lower leg, are often affected (Fröhlich et al., 2021). This is also evident in youth competitive alpine skiers around growth spurts, where the knee and the lower leg are the body regions most affected by traumatic and overuse injuries (Schoeb et al., 2020). With respect to the causes of injury, skiers' lack of physical fitness can be considered a key driver (Spörri et al., 2012). In addition, biological maturation (i.e., maturity offset) has been shown to be related to the occurrence and severity of traumatic injuries (Schoeb et al., 2020) and was found to have a moderate to strong relationship to hamstring peak force values measured during the execution of the Nordic Hamstring Exercise (NHE) (Franchi et al., 2019).

Regarding injury prevention, the hamstring muscles can act as knee stabilizers (MacWilliams et al., 1999) and have the potential to counteract the boot-induced ventral displacement of the tibia and internal rotation, as they typically occur during mechanisms leading to severe knee injuries and proximal intra-articular tibial fractures in skiers (Hasler and Hardegger, 1993; Bere et al., 2011). The hamstring muscles can also act as hip stabilizers in the case of traumatic hip injuries and may help to counteract the hip flexion moment during backward falls with the trunk bent forward, as they often occur in the immediate course of (proximal) tibia contusions or fractures (Stenroos et al., 2016). Moreover, the medial (semimembranosus and semitendinosus) and lateral (biceps femoris) hamstrings oppose external and internal rotation of the tibia, respectively (Maniar et al., 2022), which in turn may counteract rearfoot supination and rearfoot pronation during mechanisms leading to ankle sprains (Neumann, 2010). Finally, well-developed quadriceps and hamstring muscles may provide additional protection in high-energy impacts, such as those that frequently occur in ski-related femur fractures (Sterett and Krissoff, 1994).

With respect to traumatic knee injuries, the most frequent type and location of injury in youth competitive alpine skiers (Schoeb et al., 2020), the hamstrings (and in particular its lateral part, i.e., the long head of the biceps femoris (BFlh)) have a great potential to unload the anterior cruciate ligament (ACL), given its ability to counteract the internal rotation of the knee, its large capacity to generate muscle force, and its ability to generate sufficiently large posterior shear forces (Maniar et al., 2022). In addition, a recent study revealed that healthy individuals with a greater posterior-inferior directed slope of the lateral tibial plateau have increased BFlh volumes (Schmitz et al., 2017). During axial loading, a greater posterior-inferior directed slope of the lateral tibial plateau has in turn been associated with greater anterior tibial translation, greater internal tibial rotation (Beynnon et al., 2014), and increasing ACL force (Mclean et al., 2011). As both anterior tibial translation and internal tibial rotation are key components of mechanisms leading to severe knee injuries in alpine skiing and as the important functional role of the BFlh in counteracting these components is known, it is reasonable to assume that the morphology of the BFlh might be of particular interest for injury prevention.

Regarding the functional aspects of hamstring muscles typically assessed in competitive alpine skiers in the context of injury prevention, the hamstrings-to-quadriceps strength ratio (H/Q ratio) measured by an isokinetic dynamometer is probably the most well-known approach (Jordan et al., 2017; Spörri et al., 2017). This approach measures the maximal voluntary torque (MVT) during knee flexion and extension based on the hypothesis that strong hamstring muscles could prevent the anterior shift of the tibia relative to the femur during typical injury mechanisms (Jordan et al., 2017; Spörri et al., 2017). According to Johnson (1995), however, examining the peak-to-peak H/Q ratio alone is not sufficient. Based on preliminary results where seven athletes experienced an ACL injury after initial screening compared to 41 athletes who remained uninjured over the 3 years, the author proposes the assessment of the joint angle at which the hamstrings MVT results, as this was the only factor that differed significantly between the two groups. Moreover, given the timeframe in which ACL injuries typically occur (less than 60 ms) (Bere et al., 2011), it has also been proposed to complement the traditional H-Q ratio screening protocol with measurements of the rate of torque development (RTD) (Jordan et al., 2015).

In addition to assessing the functional aspects, an analysis of BFlh morphology based on ultrasound images could add a structural perspective. This could provide further valuable insights, as both joint angle-specific MVT and RTD can be associated with muscle architecture variables. Ultrasound imaging has been extensively used in both research and clinical settings to study the morphological and mechanical properties of muscle-tendon units (Sarto et al., 2021). Advanced ultrasound systems even allow the acquisition of panoramic images for muscle architecture (Noorkoiv et al., 2010) and anatomical cross-sectional area (ACSA) (Scott et al., 2012) assessments. For the assessment of muscle architecture, this is particularly advantageous for muscles with relatively long fascicles (e.g., BFlh), as otherwise a large part of the fascicle has to be extrapolated, leading to potential inaccuracies during data analysis (Franchi et al., 2020b). Moreover, in a cohort of youth competitive alpine skiers, panoramic ultrasound was recently shown to be a valid tool to measure ACSA and volume estimates for hamstring muscles when compared to MRI (Franchi et al., 2020a). However, to the best of our knowledge, there are currently no published data on BFlh architecture and the influence of BFlh morphology on the occurrence of traumatic injuries of the lower extremities in youth skiers.

Based on these considerations, the aims of the present study were twofold: 1) to describe BFlh morphology in youth competitive alpine skiers with respect to sex, age and maturity offset and 2) to investigate its association with the occurrence of traumatic injuries of the lower extremities in the upcoming season.

Materials and methods

Study design, participants and setting

The present study was designed as a cohort study with baseline measurements followed by 12-months prospective injury surveillance at 2-week intervals. 99 competitive alpine skiers voluntarily participated in the baseline measurements. All participants were recruited through announcements and information dissemination within the youth development structure of the Swiss National Skiing Association (Swiss-Ski). Eligible to participate were skiers who were members of certified regional performance centers (RLZ/RPC), i.e., the best skiers in their age group throughout Switzerland. The exclusion criteria were as follows: skiers should have not been enrolled in a back-to-sports journey after an injury and should not present systematic pathologies such as inflammatory arthritis. Based on these criteria, no participants were excluded. However, we acknowledge four dropouts during the 12-months prospective injury surveillance period, as they ended their sports career. Accordingly, a total of 95 youth competitive alpine skiers with complete datasets were included in the final analysis, of which 33 were female (mean age = 14.7 ± 0.6 years) and 62 were male (mean age = 14.9 ± 0.7 years). To investigate sex- and age-specific differences, the entire cohort was subdivided into a female and a male group, as well as into skiers under 16 years of age (U16) and skiers under 15 years of age (U15). The underlying study protocol was approved by the local ethics committee of the Canton of Zurich (KEK-ZH-NR: 2017-01395) and was conducted according to the ethical standards of the Declaration of Helsinki and national laws. All participants provided written informed consent. If they were younger than 14 years, their legal guardians signed instead.

Anthropometric measures and maturity offset estimations

The anthropometric measures included the assessment of body mass using a body scale and body height using a measuring tape to calculate the body mass index (BMI). In addition, chronological age and sex were recorded. For the estimation of biological maturation, the noninvasive method of Mirwald et al. (2002) was used. This method has already been validated for use in youth competitive alpine skiers (Müller et al., 2015). The sex-specific Mirwald formula uses the leg length (calculated from body and sitting height) and the chronological age at the time of measurement to determine the time before or after the age of fastest growth, the so-called maturity offset. The maturity offset thus reflects the difference between the time of assessment and the time when the skier is expected to reach the maximum growth rate (negative values) or the time already exceeded since reaching the maximum growth rate (positive values).

Ultrasound measurements

The ultrasound measurements were performed at the Swiss Centre for Musculoskeletal Imaging (SCMI). All ultrasound images were acquired by an experienced operator (MF) using an ultrasound device (Aixplorer Ultimate, SuperSonic Imagine, Aix-en-Provence, France). Study participants were instructed to lie prone on the massage bed with their ankles on the edge of the bed so that their feet could be kept in a neutral position and the hip and knee joints were extended. To compensate for body fluid shifts, the time between the positioning of the study participants and the image acquisition was at least 5 min, as proposed by (Perkisas et al., 2018).

An equivalent procedure for identifying and marking the region of interest (ROI), generating ultrasound images, and image analysis has been described in detail in Franchi and others (Franchi et al., 2020a; Franchi et al., 2020b). Briefly, the right posterior thigh of the study participants was first marked at 30, 40, 50, and 60% of the femur length (distance between the greater trochanter and distal end of the lateral femoral condyle) using a permanent marker. Subsequently, at each mark, the medial and lateral borders of the BFlh were identified and marked using transversal scans. These markers served as guidelines for generating longitudinal panoramic images.

For the measurement of muscle architecture, longitudinal panoramic images were generated using a 5 cm linear transducer (SuperLinear SL18-5, SuperSonic Imagine, Aix-en-Provence, France). This involved moving the transducer from the distal to the proximal myotendinous junction in a slow, controlled manner with low pressure on the underlying tissue. During image acquisition, the orientation of the transducer was adjusted to keep as many fascicles and the superficial and intermediate aponeurosis visible as possible (please see Figure 1A for a representative scan). For the measurement of anatomical cross-sectional areas (ACSA), transversal panoramic images at 30, 40, 50, and 60% marks were generated using a 4 cm linear transducer (SuperLinear SL10-2, SuperSonic

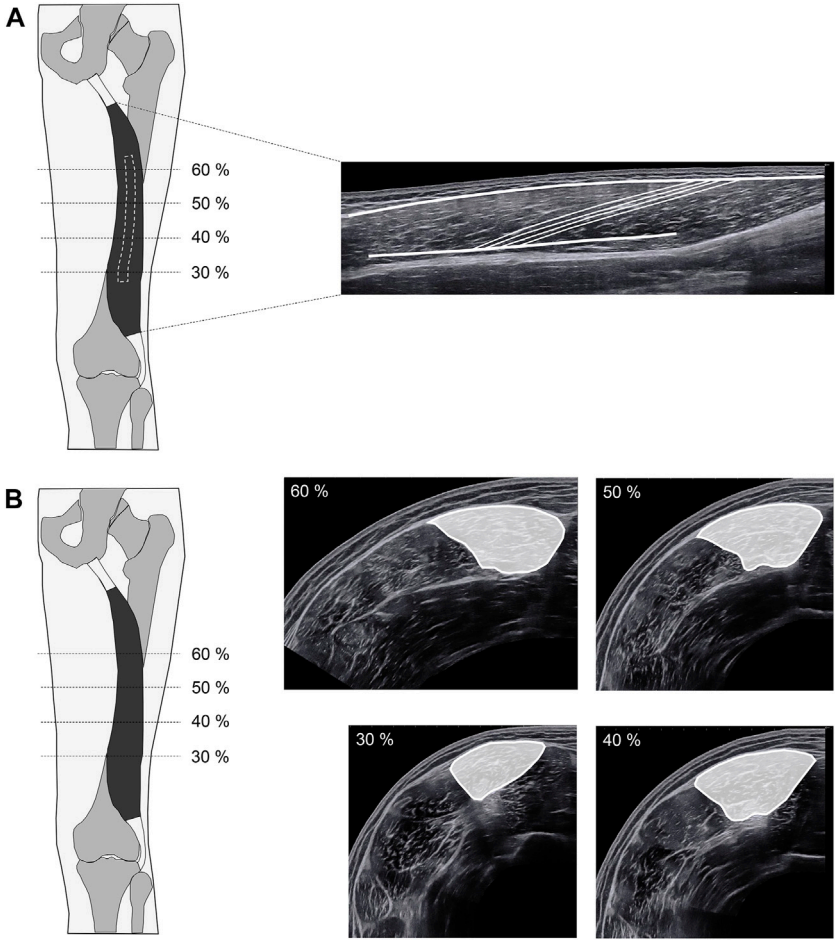

FIGURE 1
Exemplary ultrasound scans. **(A)** Longitudinal scan; **(B)** transversal scans.

Imagine, Aix-en-Provence, France). This also involved moving the transducer slowly, in a controlled manner and with low pressure from the lateral to the medial border of the BFlh. For all images, a sufficient amount of ultrasound gel was applied to the acquisition path as a conductive medium and to ensure uniform movement of the transducer.

Image analysis was performed by an experienced rater (DF) using image processing software (ImageJ, National Institutes of Health, Bethesda, MD). Figure 1A shows an example of a panoramic longitudinal scan including traced aponeuroses and fascicles. For each image, the superficial and intermediate aponeurosis and four fascicles were drawn. Muscle architecture and size measurements included fascicle length (Lf), pennation angle (PA) and muscle thickness (MT). For the statistical analysis, the respective four values for Lf, PA and MT were averaged. Figure 1B shows examples of panoramic transversal scans, including drawn ACSA of the BFlh at 30, 40, 50 and 60% of the femur length. For statistical analysis, ACSAs between 30 and 60% of the femur length were averaged (i.e., $ACSA_{avg}$) to account for the regional differences in ACSA along the femur length.

Injury surveillance

The Oslo Sports Trauma Research Centre (OSTRC) questionnaire on health problems was used for 12-months prospective injury surveillance (Clarsen et al., 2014). Self-reported data were collected and managed using the secure, web-based software platform REDCap®. The participants of the study were sent an e-mail with a personal web link to the questionnaire every second Monday. In addition, automatic reminder messages were mailed 2 days later. If the study participants did not reply within 3 days, they and their parents

TABLE 1 Overview of the participants at baseline.

	Overall (n = 95)	Female (n = 33)	Male (n = 62)	U16 (n = 37)	U15 (n = 58)
Age (y)	14.8 ± 0.6	14.7 ± 0.7	14.9 ± 0.5	15.4 ± 0.2	14.4 ± 0.3###
Maturity Offset (y)	1.2 ± 1.1	2.3 ± 0.6	0.6 ± 0.8***	1.5 ± 1.1	1.0 ± 1.1##
Body Height (cm)	166.6 ± 7.6	163.6 ± 5.8	168.2 ± 8.0**	169.1 ± 7.9	164.9 ± 7.0##
Body Mass (kg)	56.4 ± 9.1	55.5 ± 6.9	56.9 ± 10.1	59.1 ± 9.1	54.6 ± 8.8#
BMI (kg/m^2)	20.2 ± 2.2	20.7 ± 2.1	20.0 ± 2.2	20.6 ± 2.1	20.0 ± 2.2

Data are expressed as mean ± SD., Level of significance based on unpaired sample t-tests backed-up by bias-corrected accelerated (BCa) bootstrapping with 10,000 samples: ** and *** refer to a significant between-sex difference at $p < 0.01$ and $p < 0.001$, respectively. #, ## and ### refer to significant age-group differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. U16: skiers aged under 16 years; U15: skiers aged under 15 years; BMI: body mass index.

were asked to complete the questionnaire again by text message. The possibility of completing the questionnaire ended after 7 days. The self-reported health problems from the questionnaires were divided into three basic categories: illness, traumatic injury and overuse injury (Clarsen et al., 2014). Traumatic injuries were defined as those related to a clearly identifiable event (trauma), while no such triggering event could be identified for overuse injuries (Fuller et al., 2006). After completion of the 12-months prospective observation phase, all study participants were personally examined and retrospectively interviewed by an experienced sports physician (SF) to verify the accuracy of the OSTRC questionnaire data reported.

Statistical analysis

Statistical analysis was performed using statistical software (SPSS Statistics 26, IBM, Armonk, United States). To verify the normality of the distribution of any metric data, the Kolmogorov–Smirnov (KS) test, graphical techniques (i.e., histograms and quantile-quantile plots) and shape parameters (i.e., skewness and kurtosis coefficients) were used. Due to a slight departure from the distribution normality of the age variable (skewness and kurtosis values < 0.5 and < 1.2), all age-related statistical tests were backed up by bias-corrected accelerated (BCa) bootstrapping with 10,000 samples. In all other cases, standard parametric tests were applied.

Anthropometric measures and BFlh muscle morphology data are described as the mean ± SD and were tested for significant sex and age-group differences using unpaired sample t tests ($p < 0.05$). To assess the association of the four variables related to BFlh morphology with biological maturation (i.e., the predictor "maturity offset"), linear regression models were used. Finally, the association between BFlh morphology and the occurrence of traumatic injuries of the lower extremities was investigated by conducting a binary logistic regression analysis (backward LR method).

Results

Overview of anthropometric measures and maturity offset estimations in youth competitive alpine skiers

Table 1 shows an overview of anthropometric measures and maturity offset estimations of the participating youth skiers. Age did not differ significantly between the two sexes, but the group of U16 skiers was confirmed to be on average significantly older than the group of U15 skiers ($p < 0.001$). Regarding maturity offset, female skiers had higher values than male skiers ($p < 0.001$), and U16 skiers had higher values than U15 skiers ($p < 0.01$). Male skiers were on average taller than females ($p < 0.01$), and U16 skiers were on average taller than U15 skiers ($p < 0.01$). Body mass did not differ significantly between the sexes. However, U16 skiers were on average heavier than U15 skiers ($p < 0.05$). There was no significant difference in BMI between sexes or age groups.

Overview of the biceps femoris long head morphology in youth competitive alpine skiers

Table 2 shows an overview of the BFlh morphology. The two sexes did not differ significantly in the BFlh morphology variables Lf, PA, MT and $ACSA_{avg}$. U16 skiers had, on average, a larger Lf than U15 skiers ($p < 0.05$), whereas PA, MT and $ACSA_{avg}$ did not differ significantly between the age groups.

Associations between maturity offset and biceps femoris long head morphology

Figure 2 shows the results of the linear regression analyses regarding the association of BFlh morphology

TABLE 2 Overview of the biceps femoris long head morphology at baseline.

	Overall (n = 95)	Female (n = 33)	Male (n = 62)	U16 (n = 37)	U15 (n = 58)
Lf (cm)	9.2 ± 1.3	9.2 ± 1.4	9.2 ± 1.3	9.5 ± 1.3	8.9 ± 1.3#
PA (°)	11.0 ± 2.3	11.0 ± 2.5	11.0 ± 2.3	11.2 ± 2.5	10.9 ± 2.3
MT (cm)	1.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
$ACSA_{avg}$ (cm^2)	8.2 ± 1.5	7.8 ± 1.3	8.4 ± 1.6	8.5 ± 1.4	7.9 ± 1.6

Data are expressed as mean ± SD., Level of significance based on unpaired sample t-tests backedup by bias-corrected accelerated (BCa) bootstrapping with 10,000 samples: There were no significant differences between-sex differences at $p < 0.05$. # refers to a significant age-group difference at $p < 0.05$. U16: skiers aged under 16 years; U15: skiers aged under 15 years; Lf: fascicle length; PA: pennation angle; MT: muscle thickness; $ACSA_{avg}$: average anatomical cross-sectional area.

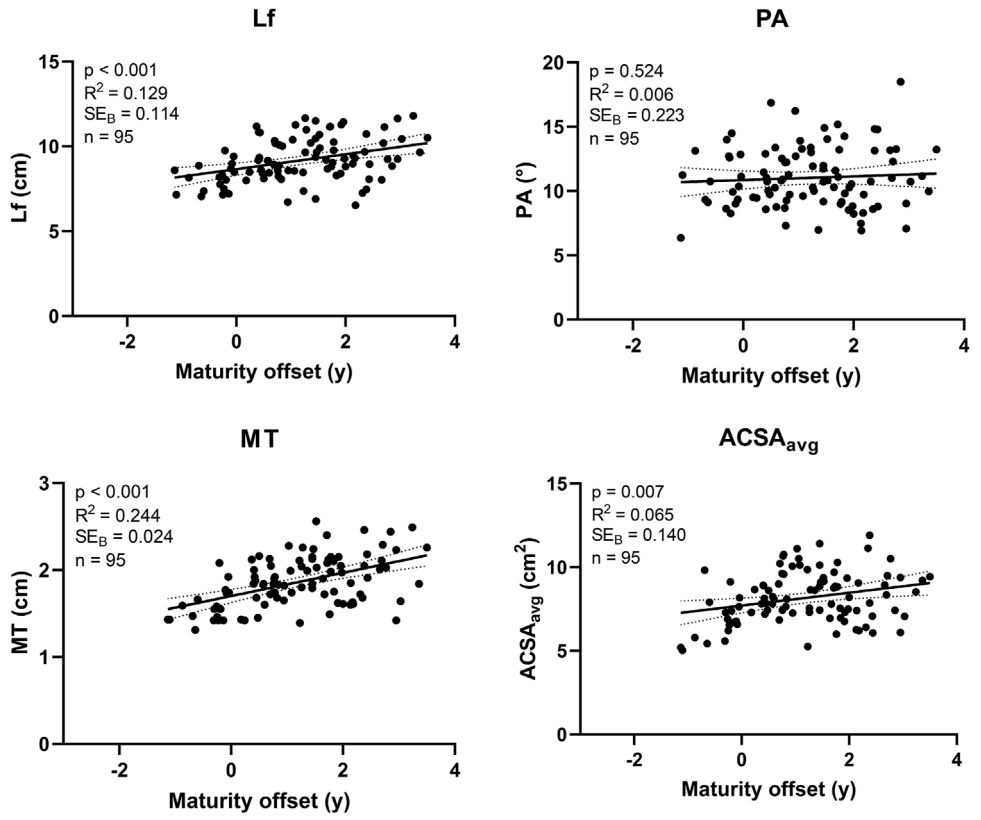

FIGURE 2
Linear regression analyses assessing the association of variables related to biceps femoris long head morphology with biological maturation (i.e., the maturity offset) in competitive alpine skiers around the growth spurt (i.e., U16 skiers). Lf: fascicle length; PA: pennation angle; MT: muscle thickness; $ACSA_{avg}$: average anatomical cross-sectional area.

with maturity offset. Lf ($p < 0.001$), MT ($p < 0.001$) and $ACSA_{avg}$ ($p = 0.007$) were found to be significantly associated with maturity offset. Lf explained 12.9% of the variance in maturity offset ($R^2 = 0.129$), while MT and $ACSA_{avg}$ explained 24.4 and 6.5% ($R^2 = 0.244$ and $R^2 = 0.065$, respectively). There was no significant association of maturity offset with PA ($p = 0.524$).

Association between biceps femoris long head morphology and traumatic injuries of the lower extremities within the subsequent season

In the subsequent season (i.e., the 12 months after the baseline measurements), a total of 37 of the 95 youth skiers

were suffering from traumatic lower extremity injuries, of which 16 sustained traumatic knee injuries (11 sprains, four contusions, one undefined trauma). Other common injuries were ankle sprains (16 affected skiers), as well as contusions or fractures at the lower leg (four skiers affected). A total of 66.7% of the injuries occurred in the first half of the year (i.e., during the competition season period from mid-November to mid-April), and 33.3% of the injuries occurred in the second half of the year (i.e., during the preseason period from mid-April to mid-November). Binary logistic regression analysis revealed a significant association between the predictor $ACSA_{avg}$ and the occurrence of traumatic lower extremity injuries in the upcoming season (Chi-square = 4.627, $R^2_{Nagelkerke} = 0.064$, $p = 0.031$, $n = 95$). If $ACSA_{avg}$ increases by one unit (i.e., 1 cm^2), the relative probability that a youth skier sustains a lower extremity injury decreases by 26.5% (Wald = 4.328, $e^B = 0.735$, $p = 0.037$). The predictors Lf, PA and MT and the potential confounder maturity offset were removed from the model by the backward LR method.

Discussion

The major findings of this study were as follows: 1) male and female skiers did not differ in BFlh morphology; 2) regardless of sex, older skiers had, on average, longer fascicles; 3) Lf, MT and $ACSA_{avg}$ were significantly associated with maturity offset, but no association was found between maturity offset and PA; and 4) $ACSA_{avg}$ was found to be associated with the occurrence of traumatic lower extremity injuries in youth competitive alpine skiers in the upcoming season.

BFlh Lf, PA, MT and $ACSA_{avg}$ did not differ between male and female skiers. One explanation for this might be found in the higher maturity offset value of the female skiers compared to the male skiers of our cohort: female skiers were on average 1.7 years more advanced in their biological maturation and had already clearly passed their growth spurt, while male skiers were still close to their age at peak height velocity. Thus, differences in BFlh morphology attributable to hormonal-related influences on muscle growth may not be fully detectable at this stage in such comparisons. Similarly, the values for PA, MT and $ACSA_{avg}$ were not significantly different between the U16 and U15 skiers. The only significant age difference was found for the Lf values, where older skiers had on average longer fascicles than younger skiers (9.5 ± 1.3 cm vs 8.9 ± 1.3 cm). Lf adaptations toward longer lengths during growth and maturation have already been shown in several studies, as highlighted in a recent review (Tumkur Anil Kumar et al., 2021). An impressive example that muscle longitudinal growth (i.e., Lf increase) can be affected by bone growth was presented in a case report by (Boakes et al., 2007). The authors investigated the change in Lf and sarcomere length and the number of vastus lateralis muscles in a 16-year-old girl who underwent a bone distraction procedure that lengthened the femur by 10%. The results showed that Lf increased from 9.1 to 19 cm during the distraction phase and then remained stable during the consolidation phase. Thus, it is plausible that growth-related changes leading to an increase in bone length as a result of physiological development may be a major driver of longitudinal muscle hypertrophy, especially in muscles of the lower extremities (Kubo et al., 2001).

Although the age-related differences in Lf in the present study are of small magnitude, they could nevertheless have functional consequences. Animal studies show that muscle fiber length can influence whole muscle maximal unloaded shortening velocity (Spector et al., 1980). In humans, however, studies that have shown relationships between Lf and functional adaptations are still scarce, although it has been shown that Lf is greater in sprinters than in distance runners (Abe et al., 2000) and is related to sprint performance in 100-m sprinters (Kumagai et al., 2000). Another functional consequence of a greater Lf may relate to the joint angle-torque curve. A training intervention based on NHE (i.e., involving lengthening muscle actions (Raiteri et al., 2021)) resulted in an adaptation toward a greater joint angle at which MVT is generated (Brockett et al., 2001).

The average values of Lf, PA and MT (9.2 ± 1.3 cm, 11.0 ± 2.3° and 1.9 ± 0.3 cm) measured across all study participants appear plausible when compared with values from panoramic ultrasound studies, which investigated youth athletes from a different sport (Lacome et al., 2019; Ritsche et al., 2021). Interestingly, when comparing these values with those of adult elite competitive alpine skiers (Lf = 8.1 ± 1.4 cm, PA = 14.9 ± 4.1° and MT = 2.1 ± 0.3 cm) reported in a previous publication from our lab (Franchi et al., 2020b), it seems that youth skiers have on average longer Lf and smaller values of PA and MT. The observation that adult elite skiers show shorter Lf values may be of particular interest because in the present study, Lf increases with age and thus during maturation. Therefore, it seems that, at least for competitive alpine skiers, once the growth spurt is completed, BFlh morphology may change toward shorter Lf and larger PA and MT. Potential explanations for such adaptations could be related to an increase in radial muscle hypertrophy (Jorgenson et al., 2020), possibly due to increased resistance training volumes.

In contrast to chronological age, where age-related differences were only observed for Lf, maturity offset had significant influences on Lf, MT and $ACSA_{avg}$. With increasing maturity offset, all the abovementioned variables of BFlh morphology increased. This was previously shown in pre, circa- and postpeak height velocity school boys, where muscle architecture variables increased from pre-to postintervention (Radnor et al., 2020). The only exception was PA, for which no association with maturity offset was found. In the literature, an increase in PA is described as a consequence of radial muscle fiber hypertrophy as a kind of "packing strategy" (Gollnick et al., 1981). Hypertrophied unipennate muscles have higher PA than untrained muscles (Kawakami et al., 1993). Compared to youth

skiers, adult elite skiers (longer exposed to resistance training programs) show greater values of PA (Franchi et al., 2020b). Based on typical coaching concepts, in competitive alpine skiing, the athletic training volume (and thus appropriate resistance training stimuli) increases noticeably after completing the youth level (Läuppi and Spörri, 2014). A possible speculation is that BFlh PA may be specifically dependent on resistance training stimuli, whereas at this age, the variables Lf, MT and $ACSA_{avg}$ typically change in relation to physiological growth alone.

According to the binary logistic regression analysis, an increase in the predictor BFlh $ACSA_{avg}$ by one unit (i.e., 1 cm^2) decreased the relative probability that a youth skier sustains a lower extremity injury during the upcoming 12 months by 26.5%. This suggests that the $ACSA_{avg}$ of the BFlh mid-belly (i.e., 30–60% of femur length) is a potentially relevant variable in the context of traumatic lower extremity injuries in youth competitive alpine skiers. The injuries occurring in this study mainly included traumatic knee injuries (sprains and contusions), ankle sprains, and contusions or fractures of the lower leg. As already explained in more detail in the introduction section, BFlh stabilizes the knee joint and has the greatest ability to protect the ACL, as it is capable of counteracting internal rotation of the knee, generating large force magnitudes, and opposing the anterior shear force (Maniar et al., 2022). Regarding ankle sprains, the BFlh opposes internal rotation of the tibia (Maniar et al., 2022), which in turn may counteract rearfoot pronation during typical mechanisms leading to ankle sprains (Neumann, 2010). In addition, the BFlh may counteract the hip flexion moment during backward falls with the trunk bent forward, as often occurs in the immediate course of tibial contusions or fractures (Stenroos et al., 2016). Finally, it should be emphasized that $ACSA_{avg}$ appears to play a superior role compared to the muscle architecture variables, as these were removed from the regression model using the LR backward method. $ACSA_{avg}$ can be considered a more global approximation for the overall muscle's strength capacity (because it covers the ACSA between 30 and 60% of the femur length and thus a large part of the BFlh) and possibly a more clinically relevant structural measure of BFlh than, for example, the local measurements of Lf, PA and MT.

Although conclusions about the functional consequences of differences in BFlh morphology are purely speculative, a larger BFlh $ACSA_{avg}$ could functionally contribute to a higher MVT and RTD. The ability to produce a high MVT and RTD is related to neuronal and muscular factors. It is well accepted that MVT is related to muscle size and that ACSA seems to be an adequate predictor of MVT (Blazevich et al., 2009). Furthermore, it is known that the MVT correlates with the RTD (Mirkov et al., 2004), whereby the correlation increases from the time of force production onset (Andersen and Aagaard, 2006). It is therefore speculated that factors that influence MVT (i.e., ACSA) can also influence RTD (Maffiuletti et al., 2016). Given that the timeframe in which ACL injuries typically occur is less than 60 ms (Bere et al., 2013), it stands to reason that both functional capacities (i.e., MVT and RTD) of the BFlh may be relevant for the prevention of traumatic lower extremity injuries in alpine skiers. In the context of ACL injuries in elite alpine ski racers, for example, Jordan et al. (2015) concluded that the assessment of MVT and RTD of hamstrings and quadriceps muscles are important determinants in a comprehensive strength assessment.

Study limitations and methodological considerations

The present study has some limitations that one should be aware of when interpreting its findings. First, although around the growth spurt, the maturity offset can be estimated with proven validity using the Mirwald formula, the estimation accuracy decreases with increasing deviation from the 0 point (i.e., the age at peak height velocity) in both positive and negative directions. The maturity offset values collected in the present study tend to be above the zero point and for female ski racers slightly outside the recommended limit of −1 to +1. Second, the data collected via the OSTRC questionnaire were self-reported by the skiers. Thus, the quality of the data strongly depends on the answers provided. To ensure sufficient data quality, skiers were assisted by their parents in answering the prospective surveys and were retrospectively interviewed by an experienced sports physician. Third, the ultrasound-based assessment of muscle morphology and associated manual evaluations are dependent on the operator/evaluators. Adequate training is therefore essential for the measurement and analysis to ensure reliability. A high reliability of the same operators/evaluators who conducted the current study has already been reported in another study and can be assumed to be on the same order of magnitude for the current study (Franchi et al., 2020a; Franchi et al., 2020b). Fourth, given the multifactorial system of injury causation, there may have been some risk of bias from unknown confounders, a circumstance that certainly limits the ability to draw conclusions about cause and effect. Nonetheless, BFlh $ACSA_{avg}$ showed a significant association with traumatic lower extremity injuries and, therefore, can be considered a meaningful proxy measure. Moreover, the experimentally determined relationship between BFlh $ACSA_{avg}$ and traumatic lower extremity injuries is also very plausible from a theoretical/biomechanical point of view, as already outlined above. Fifth, during the 12-months prospective injury surveillance, some dynamic changes in BFlh morphology may have emerged. Thus, by the time of injury, BFlh $ACSA_{avg}$ could have changed and may have slightly differed from the assessment at baseline. However, given the restricted sample size when investigating youth competitive alpine skiers, a certain period of time is required to collect a sufficient number of injury cases to ensure that the study is not underpowered.

Conclusion

The present study revealed no differences in BFlh morphology between the sexes. However, our results illustrate that in youth competitive alpine skiers, Lf, MT and $ACSA_{avg}$ can be influenced by age and biological maturation. In contrast, no influence on PA was found. In comparison to adult elite alpine skiers (Franchi et al., 2020b), youth skiers in the present study display on average longer Lf but smaller PA and MT. Accordingly, an interesting future research question would be how the resistance training stimulus should be modulated to achieve radial (increase in PA) and longitudinal (increase in Lf) muscle fiber hypertrophy and resulting functional capacities in long-term development. Furthermore, it is worth highlighting that based on the findings of the present study and those of an earlier report (Franchi et al., 2019), biological maturation can influence both the structural (BFlh morphology) and functional dimensions (the measured hamstring peak force value during the execution of NHEs). Finally, the results of the present study further support the important role of the hamstring muscles as a relevant modifiable variable for the purpose of injury prevention, and $ACSA_{avg}$ is a meaningful proxy measure that is associated with the occurrence of traumatic lower extremity injuries.

Data availability statement

The datasets presented in this article are not readily available because their access is restricted to protect the interests of the project partner Swiss-Ski and their athletes. Requests to access the datasets should be directed to joerg.spoerri@balgrist.ch.

Ethics statement

The studies involving human participants were reviewed and approved by the local ethics committee of the Canton of Zurich (KEK-ZH-NR: 2017-01395). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JS and MF conceptualized and designed the study. JS recruited the participants and organized the data collection. MF, SF, DF, and JS collected the data. DF, SF, and MF processed the data, and JS performed the statistical analysis. All authors substantially contributed to the interpretation of the data. DF, MF, and JS drafted the present manuscript; all authors revised it critically, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The optimal exercise modality and intensity for hemodialysis patients incorporating Bayesian network meta-analysis and systematic review

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Background: Physical inactivity is highly prevalent in patients with hemodialysis, and a large body of evidence reported the positive effect of different exercise modalities on their health outcomes. However, the effective dosage of exercise for hemodialysis patients still requires verification.

Objective: We aimed to determine the most effective exercise intensity and modality for improvements in physical function, blood pressure control, dialysis adequacy, and health-related quality of life for hemodialysis patients.

Design: Systematic review with network meta-analysis of randomized trials.

Data sources: Five electronic databases (PubMed, EMBASE, Web of Science, Cochrane CENTRAL, and Scopus) were searched for randomized controlled trials. Data extraction and quality appraisal were conducted by two authors independently. Data were analyzed by the R (version.3.6.2) and the Stata (version.15.0).

Result: We included 1893 patients involving four exercise modalities and six exercise intensities. Combined training (aerobic exercise plus resistance exercise) has been the top-ranking exercise modality for improving the 6-min walk test (6MWT) (surface under the cumulative ranking curve analysis (SUCRA) score, 90.63), systolic blood pressure control (SUCRA score, 77.35), and diastolic pressure control (SUCRA score, 90.56). Moreover, the top-ranking exercise intensity was moderate–vigorous for 6MWT (SUCRA score, 82.36), systolic blood pressure (SUCRA score, 77.43), and diastolic blood pressure (SUCRA score, 83.75). Regarding dialysis adequacy and health-related quality of life, we found no exercise modality or intensity superior to the placebo.

Abbreviations: ACSM, American College of Sports Medicine; RCT, randomized clinical trial; 6MWT, 6-min walk test; AT, aerobic training; RT, resistance training; CT, combined training; EMS, electrostimulation; CN, control group; BP, blood pressure; L, light intensity; LM, light–moderate; M, moderate; MV, moderate–vigorous; V, vigorous; A, according to the patient's needs; MH, mental health; PH, physical health; MD, mean difference.

Conclusion: This network meta-analysis indicated that combined training and moderate–vigorous intensity might be the most effective interventions to improve 6MWT and blood pressure control. This finding helps further guide clinical exercise prescriptions for hemodialysis patients.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/], identifier [CRD42021268535].

KEYWORDS

hemodialysis, blood pressure control, network meta-analysis, dialysis efficiency, exercise dosage

1 Introduction

Chronic kidney disease (CKD) is one of the most prevalent global health problems (Collaboration, 2020). The number of CKD patients transitioning to maintenance hemodialysis (HD) has increased in recent years (Yang et al., 2021). Nearly 25 million patients required dialysis therapy in 2020, which is expected to double by 2030 (Liyanage et al., 2015). Hemodialysis has been considered a standard alternative treatment for patients with kidney failure (O'Hare et al., 2003), but it comes along with a higher prevalence of cardiovascular diseases (Ahmadmehrabi and Tang, 2018) and mortality (Robinson et al., 2014). Regular exercise among HD patients was associated with lower mortality risk (Tentori et al., 2010). However, HD patients usually choose a sedentary lifestyle (Stack and Murthy, 2008). The Dialysis Outcomes and Practice Patterns Study (DOPPS), which consisted of 20,920 HD participants in 12 countries, showed that 43.9% never exercised and only 5.7% exercised four to five times per week (Tentori et al., 2010).

Many physiological factors contribute to physical inactivity in HD patients. First, over 80% of HD patients had chronic obstructive pulmonary diseases (Plesner et al., 2016), and cardiovascular diseases (CVDs) exist in nearly 50% of HD patients (Ahmadmehrabi and Tang, 2018). Since cardiac and pulmonary dysfunction significantly reduces absolute ventilation, HD patients can hardly increase ventilation in response to activity (Fedeli et al., 2017; Rangaswami et al., 2019; McGuire et al., 2020). Second, the prevalence of sarcopenia among HD patients was up to 65% (D'alessandro et al., 2018). HD-related sarcopenia was associated with reduced exercise capacity (Hirai et al., 2016; Kirkman et al., 2021). Third, malnutrition, anemia, and iron deficiencies were common complications of HD patients, lowering the oxygen-carrying and aerobic capacity (Hannan and Bronas, 2017; Macdougall, 2017). Other factors related to HD patients' exercise intolerance include fear of injuries, symptoms of debilitation, and fatigue (Delgado and Johansen, 2012; Wang et al., 2020).

Previous evidence demonstrated that exercise could improve cardiovascular function, physical function, and quality of life and relieve restless legs syndrome, muscle cramping, and fatigue for HD patients (Ferrari et al., 2020; Hargrove et al., 2021). Most systematic reviews have compared the control group with different exercise modalities (e.g., aerobic training, resistance training, and aerobic exercise plus resistance training) on physical function, cardiovascular health, and hemodialysis efficiency (Sheng et al., 2014; Ferreira et al., 2019; Ferrari et al., 2020). Some systematic reviews showed that resistance training (RT) and electrostimulation (EMS) could improve the 6-min walk distance (Ferrari et al., 2020). The 6MWT was one of the most commonly used tools for evaluating exercise capacity and endurance (Vogiatzaki et al., 2022). The change of 6MWT was also considered a sensitive indicator for assessing the effectiveness of an exercise intervention on hemodialysis patients (Kono et al., 2014). Aerobic training (AT) can improve HD efficiency (Ferreira et al., 2019). Another systematic review demonstrated that RT and combined training (CT: aerobic exercise plus resistance training) has no effect on 6MWT, only AT can improve 6MWT, and exercise has no effect on blood pressure control (Huang et al., 2019). Although these results have verified the effectiveness of exercise among HD patients, they are controversial. Few studies have compared the effect of different modalities on HD patients. Only one network meta-analysis (Scapini et al., 2019) compared the effect of three exercise modalities simultaneously, but the heterogeneity was high in this systematic review. As an attractive strategy, EMS was less investigated. We have no idea which specific exercise modality is appropriate for patients.

There is also a dose–response relationship between exercise intensity and health outcomes for HD patients. First, some trials showed that compared to those with low exercise intensity, higher intensity could contribute to improvements in cardiovascular outcomes among hemodialysis patients (Tzvetanov et al., 2014; Greenwood et al., 2015; Chan et al., 2018). Some trials showed that moderate and vigorous exercise could improve aerobic capacity and health-related quality of life and lower blood pressure (Reboredo et al., 2011). On the other hand, "morphologic muscle threshold" might explain the phenomenon that exercise does not affect hemodialysis efficiency (Parsons et al., 2006; Pellizzaro et al., 2013). Exercise must exceed a certain threshold to increase enough

muscle blood flow and enlarge the capillary surface and then contribute to circulating toxins transferred to the intravascular compartment and removed by dialysis (Brown et al., 2018; Andrade et al., 2019). However, no systematic review or meta-analysis is available to compare the effect of various training intensities on HD patients' health outcomes.

Due to variations in evidence and limited guidelines, it was an ongoing challenge for dialysis clinicians to support structured exercise programs as routine care (Salhab et al., 2019). Most dialysis physicians (85%) and nurses (83.3%) had no experience with interventional exercise programs for HD patients (Michou et al., 2019). The development of exercise programs has been largely overlooked for HD patients (Regolisti et al., 2018; Lambert et al., 2022) and falls far behind that of other chronic diseases (obesity, diabetes, stroke, and hypertension), for which the consensus for exercise prescription or guideline had already been established (Hansen et al., 2018; Kim et al., 2019; Teich et al., 2019; Alpsoy, 2020).

To our knowledge, there was little evidence about the optimal exercise modality and intensity for HD patients. Specific exercise prescriptions for hemodialysis patients should be designed by physical therapists and facilitate physical exercise for HD patients. To address the knowledge gap, we conducted a Bayesian network meta-analysis combining all available direct and indirect evidence across trials to compare the effect of various exercise modalities and intensities. Our research question for this systematic review was as follows: which specific exercise modality and intensity is the optimal exercise intervention to improve physical capacity, dialysis efficiency, blood pressure control, and health-related quality of life?

2 Methods

2.1 Literature search and selection criteria

The network meta-analysis was conducted in agreement with the PRISMA network meta-analysis (PRISMA-NMA) (Hutton et al., 2016), and it has been registered in the PROSPERO database (registration number ID: CRD42021268535).

We searched PubMed, EMBASE, Web of Science, Cochrane CENTRAL, and Scopus (from their inception date to 12 June 2022). The following keywords or combinations were used: exercise, aerobic exercise, resistance exercise, electrostimulation, hemodialysis, and chronic kidney disease. The complete search strategy used in PubMed is shown in Supplementary Appendix S1. Trials were also included by manually searching the reference lists of relevant reviews. We have no restrictions on the language of the publications.

We screened all included RCTs according to the criteria of the PICOS (participants, interventions, comparators, outcomes, and study design). The population group of interest was adults (≥18 years) with a chronic renal disease requiring hemodialysis. Interventions are exercise training such as aerobic training (AT), resistance training (RT), combined training (CT), and electrostimulation (EMS). The control group means the non-exercise training group. We included studies that have at least one of the outcome measures: physical function (6-min walk distance) and blood pressure (systolic blood pressure and diastolic blood pressure). The 6MWT is inexpensive, safe, and easier to perform and repeat, even though it is less sensitive to detect physical performance changes than the cardiopulmonary exercise test (Galie et al., 2016). The American Thoracic Society guideline advised that (Uszko-Lencer et al., 2017) the 6MWT was the gold standard tool to assess functional exercise capacity and activities of daily living, and moreover, it is better tolerated for chronic diseases than other walk tests (such as some tests needed to reach a speed). The 6MWT has been widely validated to assess exercise capacity in kidney diseases, including end-stage kidney disease (Shi et al., 2017), kidney transplant candidates (Cheng et al., 2020), and non-kidney solid organ transplantation (Kobashigawa et al., 2019). A long cohort also showed that compared to other physical performance assessment tools, only 6MWT was a significant predictor of severe mortality for hemodialysis patients (Vanden Wyngaert et al., 2022). Blood pressures were measured when patients were at rest. HD efficiency was measured using single-pool Kt/V (Churchill and Patri, 2021), the most common measurement for dialysis adequacy worldwide. "K" represents dialyzer urea clearance, "t" means the duration time of a single dialysis session, and "V" is the volume of urea distribution that is equal to total body water (Gotch et al., 2000). The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommended that single-pool Kt/V should be 1.2 or higher (Daugirdas et al., 2015). Moreover, mental- or physical health-related quality of life was measured by the short-form 36 health questionnaire (SF-36), which consisted of physical and mental component dimensions (Kraus et al., 2016). Physical health consisted of four scales (physical function, role physical, bodily pain, and general health), and mental health included four scales (vitality, social functioning, role-emotional, and mental health), and higher scores mean better health status (Turkmen et al., 2012).

2.2 Data abstraction

Two authors extracted data from the included trials (Yangyang Song and Lei Chen). The following information was extracted: year of publication, basic characteristics of patients, and details of intervention (exercise modality, intensity, frequency, and duration). The same two authors independently used the Cochrane Collaboration tool (Sterne et al., 2019) to assess the quality of each included trial and included randomized sequence generation, allocation concealment, performance bias, detection bias, incomplete outcome data, selective reporting, and other biases. Any

disagreements were resolved by discussion with the third author (Hongli Jiang).

The trials with AT, RT, and CT were summarized into five intensities: light, light–moderate, moderate, moderate–vigorous, and vigorous, according to ACSM recommendations (Garber et al., 2011; Scharhag-Rosenberger et al., 2015). The indicators of exercise intensity are HR (heart rate), HRR (heart rate reserve), and VO_{2max} or some subjective parameters such as the rating of perceived exertion (RPE). The resistance exercise was classified by repetition maximum (RM). We added an intensity according to the patient's need, which means there was no specific exercise velocity or target heart rate for patients to achieve (Molsted et al., 2004; Pomidori et al., 2016). The classification details of the intensity of each exercise are shown in Supplementary Appendix S2 eTable1. The summarization process was performed by two authors independently. We contacted the authors when the data were unavailable.

2.3 Synthesis analysis

We performed a pairwise meta-analysis using a random-effect model. The mean difference (MD) and a 95% confidence interval (CI) were used as effect estimates among different studies. Heterogeneity was assessed by I^2 statistics, 0–40%, 40%–60%, 60%–75%, and 75–100%, which indicated low, moderate, substantial, and considerable heterogeneity, respectively (Hutton et al., 2016). The unit-of-analysis error may exist in studies with two or more experimental groups. To overcome this error, we split these trials into two or more groups, with one control group in the pairwise meta-analysis (Andreato et al., 2019).

We performed NMA using a Bayesian framework noninformatively prior to distributions and the Markov chain Monte Carlo method (MCMC). The Bayesian network meta-analysis can provide evidence from direct and indirect comparisons. The model allows comparisons of the different treatments simultaneously. The network plot was generated to represent the connection between each treatment. The lines in the plot represented direct comparisons between two treatments, the width of the lines represented the number of trials, and the size of each node represented the number of participants (Salanti et al., 2011). We ranked the treatments using the surface under the cumulative ranking curve (SUCRA). SUCRA reported the cumulative ranking probabilities and considered the more precise estimation of ranking probabilities (Mbuagbaw et al., 2017). The value of SUCRA close to 1 represented the best intervention (Salanti et al., 2011). We presented the treatment effect and the corresponding 95% CI by league tables.

For each outcome, heterogeneity was evaluated using the I^2 statistic. The node-splitting approach was used to examine whether there exists any inconsistency between direct and indirect evidence, and the *p*-value was higher than 0.05, which indicated no significant inconsistency. We would analyze using clinical factors if there does exist statistical inconsistency and heterogeneity. Sensitive analysis was performed by excluding the high risk of trials. We also judged publication bias by inspecting the asymmetry of a comparison-adjusted funnel plot and the *p*-value of Egger's test. Serial analyses were performed in STATA (Stata version 15.0), R language, and the "RJAGS" package.

Our analyses were based on previously published trials, and there is no need for ethical approval and patient consent.

3 Results

3.1 Flow of studies through the review

The flow diagram of the included studies is presented in Figure 1. The initial search yielded 4,179 records, and 12 studies were included in the reference lists of published systematic reviews. A total of 780 duplicates were excluded, and 3,411 studies were excluded after abstract screening. A total of 182 studies were retrieved in full text for further consideration. In total, 46 trials were finally included in the qualitative analysis.

3.2 Study characteristics and risk of bias assessment

The 46 studies comprised 1893 participants, and the study duration ranged from 8 to 40 weeks. Among the studies included, 1,011 were allocated to exercise training interventions, and 882 were allocated to the non-exercise group. Exercise training interventions included aerobic (n = 417), resistance (n = 282), and combined aerobic and resistance exercise (n = 252), EMS (n = 60).

Nineteen studies compared the effects of AT and the control group (Painter et al., 2002; Tsuyuki et al., 2003; Sakkas et al., 2008; Toussaint et al., 2008; Koh et al., 2010; Reboredo Mde et al., 2010; Wilund et al., 2010; Giannaki et al., 2013; Mohseni et al., 2013; Wu et al., 2014; Groussard et al., 2015; Liao et al., 2016; Pomidori et al., 2016; Cooke et al., 2018; Fernandes et al., 2019; Lin et al., 2021; Perez-Dominguez et al., 2021; Kim et al., 2022; Vogiatzaki et al., 2022). Nine studies evaluated RT and the control group. (Cheema et al., 2007; Pellizzaro et al., 2013; Kirkman et al., 2014; Abreu et al., 2017; Rosa et al., 2018; Dong et al., 2019; Martins do Valle et al., 2020; Gadelha et al., 2021).

Ten studies evaluated CT (DePaul et al., 2002; Molsted et al., 2004; van Vilsteren et al., 2005; Ouzouni et al., 2009; Frih et al., 2017; Huang et al., 2019; Hatef et al., 2020; Yeh et al., 2020; Assawasaksakul et al., 2021; Myers et al., 2021), and three studies evaluated EMS (Roxo et al., 2016; Schardong et al., 2017; Suzuki et al., 2018). In total, 35 studies were two-arm studies, three were three-arm studies (Afshar et al., 2010; Dobsak et al., 2012; McGregor et al., 2018), and two trials were four-arm studies (Kopple et al.,

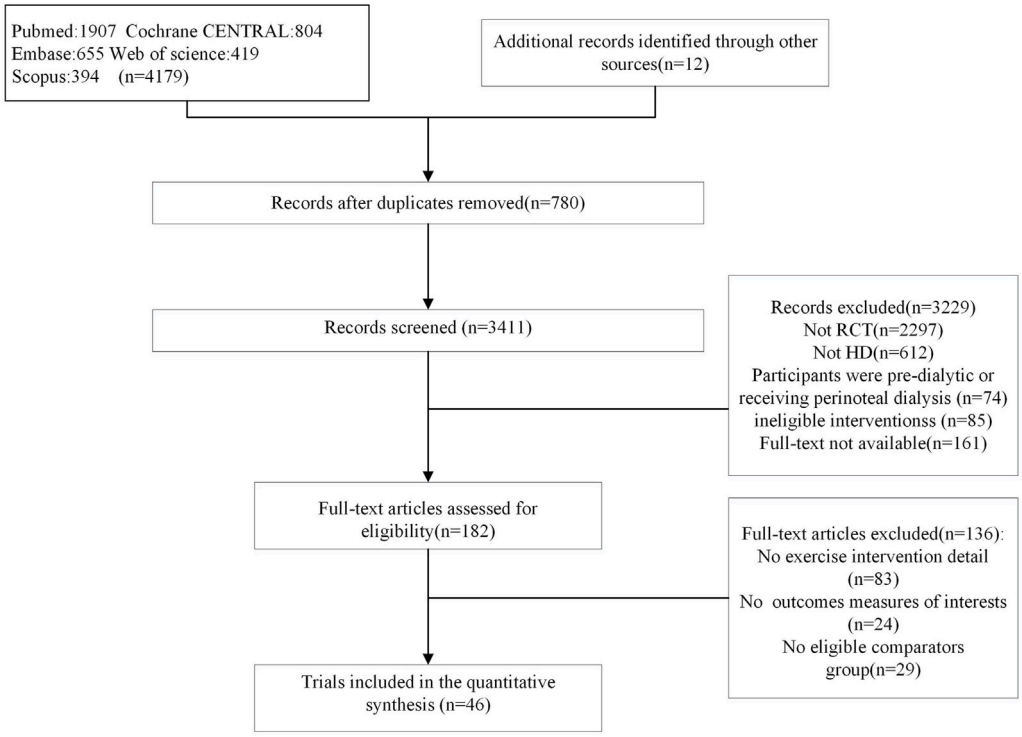


FIGURE 1
Flow of studies through the review.

2007; Thompson et al., 2016). The duration of most trials ranged from 8 weeks to 12 months. The exercise duration was 8–12 weeks in 26 trials, 12–24 weeks in 11 trials, and ≥24 weeks in nine trials. The frequency of exercise training per week ranged from 2 to 4 times except for one study, which was performed once per day (Myers et al., 2021). The exercise duration for most trials per session was 30–90 min. The detailed characteristics of included trials are presented in Supplementary Appendix S3 eTable2.

The risk of bias assessment for each study is shown in Supplementary Appendix S4. Most trials are not of high methodological quality. As the included studies involved exercise interventions, it may be difficult to blind patients and investigators, and most trials tend to have unclear risks of performance bias, and eight studies have high risks of detection bias. Random sequence generation was reported in only 20 studies (43.48%), and 26 studies (56.52%) had unclear risks. Allocation concealment in 13 studies (28.26%) was reported, while 32 studies had unclear bias (69.57%). Nearly half of the studies did not report incomplete outcome data (50%). Only ten trials exhibited a low risk of selective outcome reporting (21.73%), and 33 studies (71.74%) had an unclear bias. Twenty studies (43.48%) were judged high or unclear in the domain risk of other bias since they did not report the sample size calculation. It was challenging to explain the result, especially in some studies with small sample sizes.

3.3 Pairwise meta-analysis

The detailed results of the pairwise meta-analysis are shown in Supplementary Appendix S5 eTable3. For 6MWT, CT (MD = 4.9, 95% CI = 3.1 to 6.7, I^2 = 0%), AT (MD = 3.3, 95% CI = 0.7 to 6.0, I^2 = 0%), and RT (MD = 2.5, 95% CI = 0.56 to 4.5, I^2 = 0%) reached statistical significance than the control group. However, EMS had limited effect on 6MWT (MD = 2.3, 95% CI = −2.1 to 6.6, I^2 = 0%). Moderate–vigorous intensity (MD = 4.5, 95% CI = 1.9 to 7.1, I^2 = 4.8%) is superior in the control group. Moderate (MD = 5.0, 95% CI = 1.80 to 7.7, I^2 = 0%) and moderate–vigorous intensity (MD = 4.6, 95% CI = 0.80 to 8.4, I^2 = 0%) are more efficient than light intensity. Regarding Kt/V, no exercise modality and intensity has got statistical significance improvement than the control group.

As for systolic and diastolic blood pressure, no exercise modality significantly reduced systolic or diastolic blood pressure more than the control group. Moderate–vigorous exercise significantly reduced systolic blood pressure (MD = −8.7, 95% CI = −17 to −1.6, I^2 = 70.8%) and diastolic blood pressure (MD = −4.9, 95% CI = −9.9 to −0.35, I^2 = 74.2%) than the control group.

For mental health-related quality of life, CT (MD = 3.6, 95% CI = −4.1 to 11.0, I^2 = 58.8%), AT (MD = 3.8, 95% CI = −2.2 to 10.0, I^2 = 60.0%), and RT (MD = 5.7, 95% CI = −5.4 to 17.0, I^2 = 70.3%) did not reach statistical significance than the control

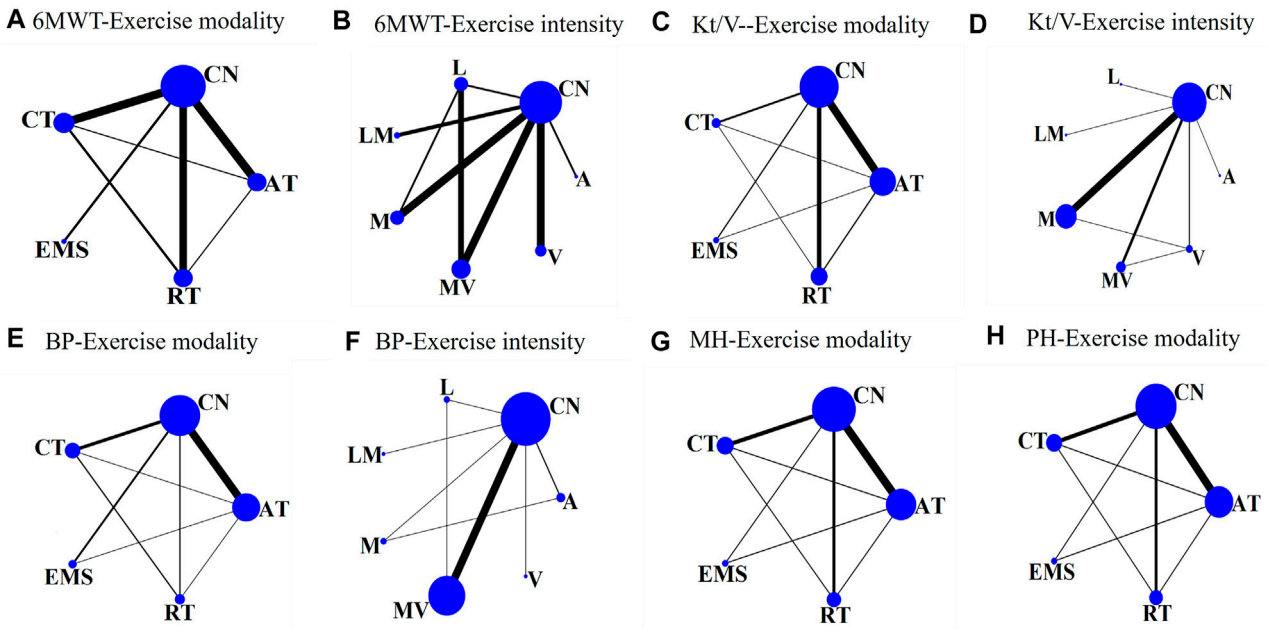


FIGURE 2
Network plots of clinical trials for hemodialysis patients comparing different exercise modalities or intensities to **(A)**: 6MWT-exercise modalities. **(B)**: 6MWT-exercise intensities. **(C)** Kt/V- exercise modalities. **(D)**: Kt/V-exercise intensities. **(E)** blood pressure-exercise modalities. **(F)**: blood pressure-exercise intensities. **(G)**: Mental health-exercise modalities. **(H)** Physical health-exercise modalities.

group. Moderate (MD = 3.6, 95%CI = −7.6 to 16.0, I^2 = 84.0%) and moderate–vigorous intensity (MD = 2.4, 95% CI = −4.6 to 9.4, I^2 = 66.6%) is not superior to that in the control group. For physical health-related quality of life, CT (MD = 3.6, 95% CI = −5.9 to 13.0, I^2 = 0%), AT (MD = 6.9, 95% CI = −23.0 to 11.0, I^2 = 77.4%), and RT (MD = 8.7, 95% CI = −3.0 to 21.0, I^2 = 70.3%) did not reach statistical significance than the control group. Moderate (MD = −0.52, 95% CI = −12.0 to 11.0, I^2 = 0%) and moderate–vigorous intensity (MD = 5.8, 95% CI = −1.2 to 13.0, I^2 = 82.9%) is not superior to that in the control group. However, only one trial (Dobsak et al., 2012) compared the effect of intra-dialytic EMS (20 weeks) on health-related quality of life among HD patients, and significant improvement was observed in the mental function (p = 0.001) and physical function (p = 0.006) compared to that in the control group.

3.4 Synthesis results of network meta-analysis

Our network analysis of exercise modality was conducted among the four treatments. EMS was difficult to classify based on intensity. Only the studies on AT, RT, or CT were included to estimate the effect of different intensities. The visual network plot was performed to display evidence among different exercise modalities and exercise intensities. All network plots are presented in Figure 2. Each node represented a unique intervention or control group. The lines showed a direct relationship between interventions, and the width was weighted according to the number of trials between them. Figure 3 presents the interventions of mean difference and the 95% credible interval (CrI) in accordance with the control group. Supplementary Appendix S6 illustrates the effect sizes (MD) for all exercise modalities or intensities. Figure 4 shows the corresponding surface under the cumulative ranking curve analysis (SUCRA) in terms of different outcomes.

3.4.1 Assessment of heterogeneity and inconsistency for each outcome

There was no significant statistical heterogeneity (I^2<10%) for each exercise modality and intensity; more details are shown in Supplementary Appendix S7 eTable4. The assessment of incoherence global results for each outcome is presented in Supplementary Appendix S8 eTable5. The node split model results indicated no difference between direct and indirect outcomes (p values >0.05). The node-splitting results are presented in Supplementary Appendix S8 eTable6.

3.4.2 Network meta-analysis results for 6MWT

Twenty studies that assessed the effect of different exercise modalities on 6MWT were eligible for network analysis. A total of five unique treatments were included in the network plot (Figure 2A). CT (MD = 4.89; 95% CI, 3.02–6.60), RT (MD = 2.50;

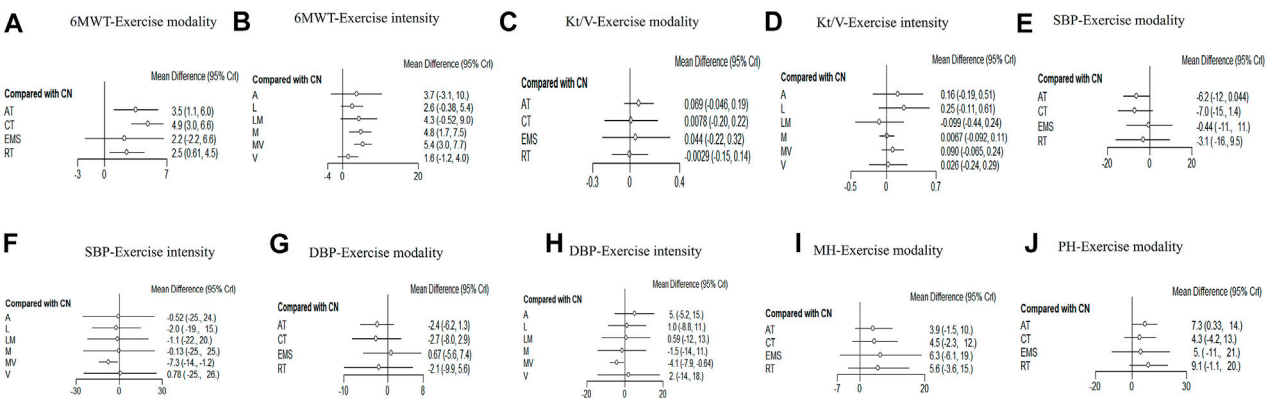
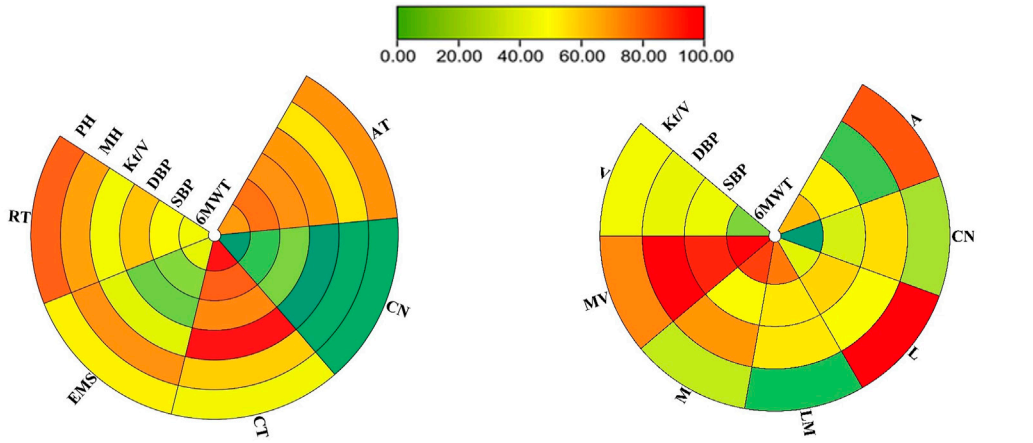


FIGURE 3
Forest plots for each outcome category represent the comparisons of the active intervention with no intervention (control group). **(A)**: 6MWT-exercise modalities and **(B)**: 6MWT-exercise intensities. **(C)** Kt/V-exercise modalities. **(D)**: Kt/V-exercise intensities. **(E)** Systolic blood pressure-exercise modalities. **(F)**: Systolic blood pressure-exercise intensities. **(G)**: Diastolic blood pressure-exercise modalities. **(H)**: Diastolic blood pressure-exercise intensities. **(I)** Mental health-exercise modalities. **(J)** Physical health-exercise modalities.



SURCA	AT	CN	CT	EMS	RT
6MWT	66.01	4.09	90.63	43.65	45.63
SBP	73.48	21.16	77.35	30.02	47.99
DBP	67.85	28.12	69.15	26.50	58.38
Kt/V	66.49	3.98	90.56	43.28	45.68
MH	51.75	9.83	56.57	67.48	64.36
PH	67.65	11.05	45.28	49.23	76.79

SURCA	A	CN	L	LM	M	MV	V
6MWT	55.05	5.42	40.82	64.89	73.47	82.36	28.00
SBP	46.48	39.41	50.90	47.82	45.24	77.43	42.72
DBP	21.64	49.62	44.37	47.93	60.67	83.75	42.03
Kt/V	70.51	32.84	83.92	19.68	36.67	63.00	43.39

FIGURE 4
Schematic details of the treatments according to the surface under the cumulative ranking curve analysis (SUCRA).

95% CI, 0.61–4.48), and AT (MD = 3.53; 95% CI, 1.09–6.01) were more effective than those of the control group (Figure 3A). EMS (MD = 2.23; 95% CI, −2.15–6.61) had no significant effect on 6MWT than in the control group. The result showed that CT is the best treatment with the highest probability (SUCRA score, 90.63).

A total of six unique exercise intensities were included in the network plot (Figure 2B). The light intensity (MD = 2.55; 95% CI, −0.52–5.39), light–moderate intensity (MD = 4.30; 95% CI, −0.53–9.01), and "A" (the intensity was according to the patient's need) had no significant effect on 6MWT than in the control group. The moderate–vigorous intensity (MD = 5.36;

95% CI, 2.99–7.73) showed significantly superior efficacy over the control group (Figure 3B). The moderate–vigorous intensity was more efficient than the light intensity (MD = 2.82; 95% CI, 0.02–5.80) and the vigorous intensity (MD = 3.82; 95% CI, 0.39–7.44). The top-ranked interventions for 6MWT were moderate–vigorous intensity (SUCRA score, 82.36).

3.4.3 Network meta-analysis results for hemodialysis efficiency

As for Kt/V, the network plot (Figure 2C) consisted of 21 studies with five different exercise modalities. No difference has been found between the intervention and control groups (Figure 3C). CT had the highest probability (SUCRA score, 90.56) of being the best treatment for this outcome. Twenty studies assessed the effect of seven different exercise intensities for Kt/V. The network plot is shown in Figure 2D. All intensities had a 95% CrI including the zero effect (Figure 3D). Light-intensity exercise was the most effective treatment (SUCRA score, 83.92).

To evaluate the effect of intradialytic exercise on improving Kt/V, we also conducted an analysis that only included studies in which the intervention was intra-dialytic exercise. Twenty studies were included in the analysis. The results showed that even intra-dialytic exercise exhibited no significant effect on improving Kt/V. The details of forest plots are presented in Supplementary Appendix S9. AT had the highest probability (SUCRA score, 74.14) and was the best treatment for this outcome. Light-intensity exercise was the most effective treatment (SUCRA score, 83.93).

3.4.4 Network meta-analysis results for blood pressure

In the network meta-analysis of blood pressure, 21 studies on exercise modality's effects and nineteen studies on different exercise intensities were included. Only two network plots of exercise modality (Figure 2E) and exercise intensity (Figure 2F) were presented for blood pressure because all studies simultaneously measured both systolic and diastolic blood pressure.

In the network meta-analysis of systolic blood pressure, none of the exercise training modalities was more effective than the control group (Figure 3E). Moderate–vigorous exercise intensity (MD = −7.33, 95% CI, −14.08, 1.25) was more effective in reducing systolic blood pressure than in the control group (Figure 3F). CT had the highest probability (SUCRA score, 77.35) and was the best treatment for systolic pressure control. Moderate–vigorous exercise intensity (SUCRA score, 77.43) has the best intensity for systolic pressure control.

In the network meta-analysis of diastolic blood pressure, all exercise modalities were statistically equivalent to the control group (Figure 3G). CT had the probability (SUCRA score, 69.15) to be the best treatment. Moderate–vigorous exercise intensity (MD = −4.13, 95% CI, -7.93, −0.64) was more effective in reducing systolic blood pressure than in the control group. The other exercise intensities had a 95% CrI, including the zero effect (Figure 3H). The moderate–vigorous intensity (SUCRA score, 83.75) was the best for diastolic blood pressure control.

3.4.5 Network meta-analysis results for health-related quality of life

In the network meta-analysis of health-related quality of life, nineteen studies on the effect of exercise modalities and sixteen studies on different exercise intensities were included. The network plots of exercise modality (Figure 2G; Figure 2H) were presented for mental- and physical health-related quality of life.

We did not conduct a network analysis about the effect of different exercise intensities on health-related quality of life. All the included studies compared the effect of different exercise intensities with that of the control group, and no study compared the effect of various training intensities on health-related quality of life. Therefore, it was unable to form a connected network and cannot satisfy the connectivity assumption of network analysis (Higgins et al., 2012; Watt et al., 2019).

In the network meta-analysis of mental health-related quality of life, none of the exercise training modalities was more effective than those of the control group (Figure 3I). CT (MD = 4.46; 95% CI, −2.29–11.51), RT (MD = 5.62; 95% CI,−3.57–15.18), and AT (MD = 3.95; 95% CI, −1.49–10.00) were not effective compared with those in the control group (Figure 3I). EMS (MD = 6.32; 95% CI, −6.08–19.04) had no significant effect on mental health quality than in the control group. EMS had the highest probability (SUCRA score, 67.48) and was the best treatment for mental health-related quality of life.

In the network meta-analysis of physical health quality, all exercise modalities, except AT, were statistically equivalent to the control group (Figure 3J). CT (MD = 4.33; 95% CI, −4.21–13.02), RT (MD = 9.13; 95% CI, 1.12 to −19.59) had no statistical effect than the control group. AT (MD = 7.26; 95% CI, 0.33–13.8) was more effective than the control group (Figure 3J). EMS (MD = −4.95; 95% CI, −20.73 to 10.91) had no significant effect on physical health quality than the control group. RT had the probability (SUCRA score, 76.79) to be the best treatment.

3.4.6 Publication bias and sensitivity analysis for each outcome

The comparison-adjusted funnel plots were symmetrically distributed and showed no publication bias. Egger's test revealed no publication bias, and the p-value was higher than 0.05 for all outcomes, except for the exercise modality of 6MWT. There was evidence of publication bias ($p = 0.02$). The results are shown in Supplementary Appendix S10. We conducted a sensitivity analysis for all outcomes, and the results did not change when

we excluded the high-risk studies. The result is presented in Supplementary Appendix S11.

4 Discussion

The network meta-analysis method has been used to explore the optimal exercise modality or intensity in some chronic diseases, such as obesity (Chang et al., 2021) and diabetes (Schwingshackl et al., 2014). Only a network meta-analysis (Scapini et al., 2019) has compared the effectiveness of different exercise modalities (AT, RT, and CT) with placebo on physical function, blood pressure, and hemodialysis efficiency among HD patients. That review needs to be updated, and it took no account of exercise intensity. Our research provided more comparisons and trials to comprehensively assess the effect of different exercise modalities. We also added a new outcome indicator, quality of life, in the current study. More importantly, our study is the first network meta-analysis to compare the effectiveness of different exercise intensities for HD patients.

In the current study, we compared the effect of various exercise modalities and intensities on different outcomes for hemodialysis patients. Most included trials last 4–6 months (3 sessions/weeks) and 30–50 min per session. The result showed some interesting findings, and we recommended that combined training (CT) and moderate–vigorous intensity are the optimal exercise modality and intensity for improving physical function and controlling blood pressure in HD patients.

Although the method of evaluating exercise intensity (light, moderate, and vigorous) has been widely used in the previous literature (Garber et al., 2011; Scharhag-Rosenberger et al., 2015), there are no standard definitions and descriptions of moderate–vigorous or light–moderate intensity exercise. However, physical activity of moderate–vigorous intensity is commonly recommended for health benefits in health guidelines (Tremblay et al., 2011). This ambiguity also makes it difficult for practitioners to offer accurate exercise advice. In order to improve the generalization of MV, a recent review by Brian (MacIntosh et al., 2021) suggested that the rating of perceived exertion might be a practical and effective measurement to define moderate–vigorous exercise. Therefore, the current study defined the moderate–vigorous intensity as RPE at 12–16 and the light–moderate intensity as RPE at 11–13. Meanwhile, based on the previous literature (Yoshioka et al., 2021a; Yoshioka et al., 2021b), which conducted exercises on chronic kidney diseases, we defined the MV intensity as higher than 3.0 metabolic equivalents.

Evidence has shown that moderate–vigorous intensity of exercise might benefit kidney function and physical function for patients with chronic diseases. A cross-sectional study (Hara et al., 2021) of 66,603 Japanese patients demonstrated that replacing 1 h of sedentary behavior with moderate–vigorous physical activity (≥3.0 metabolic equivalents) could reduce the incidence of chronic kidney diseases by 3–4%. Some studies (Yoshioka et al., 2021a; Yoshioka et al., 2021b) demonstrated that a slight increase (10 min/day) in the time of moderate–vigorous intensity exercise (≥3.0 metabolic equivalents) contributes to maintaining skeletal muscle strength and isometric knee extension strength in patients with chronic kidney disease, especially attenuating bone density decline. It is to be noted that the amount of moderate–vigorous intensity of exercise might exert different effects. A 10-year cohort (Wang et al., 2021) of 403,681 individuals suggested that participants who performed exercise at moderate–vigorous intensity and those with 50–75% vigorous intensity exercise had 17% lower all-cause mortality than those with no vigorous exercise. Future work should focus on the amount of moderate–vigorous intensity exercise and whether the proportion of moderate or vigorous intensity exerts different impacts on individuals.

Our results indicated that exercise could positively affect 6MWT regardless of modality and intensity. The most remarkable improvement was observed in exercise mortality with combined exercise and moderate–vigorous intensity. This finding was in agreement with that of another traditional meta-analysis that showed that exercise could increase at least 60 m for 6MWT (Ferrari et al., 2020). The 6MWT could reflect the ability to perform daily life activities and assess the global exercise responses. The previous meta-analysis suggested that 6MWT was sensitive as VO_2 to evaluate exercise capacity and the effect of exercise among hemodialysis patients (Huang et al., 2019). Another meta-analysis (Gomes Neto et al., 2018) showed that all types of exercise improved the 6MWT distance among hemodialysis patients. It is critical to improving physical function for HD patients, characterized as 6MWT, which has been an independent mortality predictor of increased cardiovascular events (Sietsema et al., 2004). A 3-year follow-up showed that an increase of 20 walked meters in 6MWT among HD patients can reduce all-cause death by 12%, all-cause hospitalizations by 4%, and fatal and non-fatal cardiovascular events by 7% (Torino et al., 2014).

Our result showed that exercise had a limited effect on Kt/V. The result was consistent with that of previous systematic reviews (Salhab et al., 2019; Scapini et al., 2019). This might mean that exercises, regardless of modality or intensity, might not be able to improve hemodialysis efficiency or Kt/V was not the optimal measurement to evaluate the effect of exercise on hemodialysis efficiency. Kt/V represents the clearance of urea, a small solute distributed across plasma membranes (Vanholder et al., 2019). Some studies used the removal of serum potassium, creatinine, β2-microglobulin, or phosphate rather than urea to evaluate HD efficiency (Sampaio et al., 2012; Orcy et al., 2014). A meta-analysis (Ferreira et al., 2019) of three to five studies revealed that aerobic exercise could decrease creatinine levels for HD patients. Another study also found that exercise has no effect on Kt/V but had a

significant decrease in serum phosphorus, creatinine, and potassium (Pellizzaro et al., 2013). It is to be noted that a recent review demonstrated that exercise had limited effects on improved small-molecule clearance for HD patients (Kirkman et al., 2019). Since Kt/V has been used in most previous studies, we still choose Kt/V as the measurement for hemodialysis efficiency. Future trials could assess the effect of exercise on the clearance of middle molecules and protein-bound uremic toxins.

The prevalence of hypertension among HD patients was up to 70–90% (Bikos et al., 2018), and uncontrolled hypertension was strongly associated with cardiovascular diseases (Loutradis et al., 2017). Our result found that exercise modalities did not affect blood pressure control, similar to a recent meta-analysis (Huang et al., 2019), which showed that the effect size of exercise on SBP and DBP was −0.18 (−0.42, 0.07) and −0.23 (−0.69, 0.24), respectively. In contrast with our results, another meta-analysis showed that CT could significantly reduce diastolic BP, and AT can improve systolic BP (Ferrari et al., 2020). However, their research showed moderate heterogeneity (44%). Our analysis has low heterogeneity and yields more reliable results.

It is to be noted that our results showed that moderate–vigorous intensity exercise could significantly reduce systolic blood pressure (8 mmHg) and diastolic blood pressure (4 mmHg). Consistent with our results, an 8-year cohort (Diaz et al., 2015) study consisting of 1,311 participants with moderate–vigorous physical activity (≥3.5 metabolic equivalents) had lower hypertension incidence for African–Americans (hazard ratio 0.76; 95% CI 0.58–0.99) compared to those who do not perform exercise. Another research showed that a 2 mmHg decrease in systolic blood pressure could reduce coronary heart disease and stroke in adults with hypertension (Heiwe and Jacobson, 2014). These results might be helpful in management of HD patients.

Our result found that exercise did not affect the mental or physical quality of life among HD patients. Contrary to our results, a systematic review (Huang et al., 2019) suggested that an 8-week exercise regimen could reverse the reduction of physical and mental health-related quality of life (SMD = 0.34, 95% CI: 0.09–0.59 and SMD = 0.27, 95% CI: 0.02–0.51, respectively) among HD patients. However, the meta-analysis only included seven trials (139 participants), and the limited sample size might exist bias. Nevertheless, a review conducted by Gomes Neto et al. (2018) demonstrated that AT could not improve the whole quality of life for HD patients. Previous studies (Sheng et al., 2014; Chung et al., 2017) also showed that AT and RT did not have a significant difference in the mental health-related quality of life. The baseline quality of life scores might cause the discrepancy. Painter et al. (2000)showed that HD patients with baseline physical quality of life (SF-36) scores less than 34 were associated with more remarkable improvement but not found in those with scores higher than 34. We should focus on HD patients with lower baseline quality of life scores who might more easily be affected by exercise.

5 Strengths and limitations

The strength of our network meta-analysis is that it provided evidence to guide the exercise of clinical practice for HD patients, which integrated direct and indirect comparisons to compare various modalities and intensities. A significant strength is that we comprehensively estimated the effectiveness of exercise intensities for HD patients. The validated measurement was used to classify the exercise intensities. Moreover, we provided the ranking of different exercise training modalities and intensities based on health outcomes for HD patients. Our evidence can help clinicians to choose the optimal exercise modality and intensity for HD patients. Furthermore, we found no heterogeneity and inconsistency in global and local analysis.

However, there were also some limitations. Most included studies were performed in a single medical center with limited number of patients. Future trials are required to be performed in multiple centers. The included trials did not compare the duration and session of exercise, contributing to difficulty in ascertaining the optimal exercise dose within the current literature. Since most of the included studies failed to report adverse events, this study does not pay attention to the adverse effects of exercise. A meta-analysis with limited trials showed that exercises would not increase the rate of adverse events for HD patients (Chung et al., 2017). Few trials have reported the adherence of patients after the intervention. We failed to explore exercise adherence after the intervention. Clinicians should help patients develop long-term exercise habits.

6 Conclusion

The present network analysis included four exercise modalities and six exercise intensities. In conclusion, combined training was the most effective intervention among current exercise interventions, and moderate–vigorous intensity was the most effective intensity in improving 6MWT and blood pressure control. In addition, exercise might not affect Kt/V and quality of life. Clinicians could give the optimal exercise prescription even for hemodialysis patients at home. Supervision was also needed to ensure the right exercise intensity. Future research can be conducted to estimate the clinical effect of exercise duration for HD patients and provide more evidence for clinical practice.

Author contributions

All authors contributed equally to the development of the manuscript. With regard to specific roles, HJ, MW, and QH supported conceptualization of the manuscript. LC, MW, YS, and JX supported data extraction and data analysis. JX and YS

wrote the manuscript. HJ supported all phases of the manuscript's development.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.945465/full#supplementary-material

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Exercise testing and postoperative complications after minimally invasive lung resection: A cohort study

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Background: Peak oxygen uptake ($\dot{V}O_2$) during cardiospulmonary exercise testing (CPET) is used to stratify postoperative risk following lung cancer resection but peak $\dot{V}O_2$ thresholds to predict post-operative mortality and morbidity were derived mostly from patients who underwent thoracotomy.

Objectives: We evaluated whether peak $\dot{V}O_2$ or other CPET-derived variables predict post-operative mortality and cardiopulmonary morbidity after minimally invasive video-assisted thoracoscopic surgery (VATS) for lung cancer resection.

Methods: A retrospective analysis of patients who underwent VATS lung resection between 2002 and 2019 and in whom CPET was performed. Logistic regression models were used to determine predictors of postoperative outcomes until 30 days after surgery. The ability of peak $\dot{V}O_2$ to discriminate between patients with and without post-operative complications was evaluated using Receiver operating characteristic (ROC) analysis.

Results: Among the 593 patients, postoperative cardiopulmonary complications occurred in 92 (15.5%) individuals, including three deaths. Mean peak $\dot{V}O_2$ was 18.8 ml·kg^{-1}·min^{-1}, ranging from 7.0 to 36.4 ml·kg^{-1}·min^{-1}. Best predictors of postoperative morbidity and mortality were peripheral arterial disease, bilobectomy or pneumonectomy (versus sublobar resection), preoperative FEV_1, peak $\dot{V}O_2$, and peak $\dot{V}E/\dot{V}CO_2$. The proportion of patients with peak $\dot{V}O_2$ of < 15 ml·kg^{-1}·min^{-1}, 15 to < 20 ml·kg^{-1}·min^{-1} and ≥ 20 ml·kg^{-1}·min^{-1} experiencing at least one postoperative complication was 23.8, 16.3 and 10.4%, respectively. The area under the ROC curve for peak $\dot{V}O_2$ was 0.63 (95% CI: 0.57–0.69).

Conclusion: Although lower peak $\dot{V}O_2$ was a predictor of postoperative complications following VATS lung cancer resection, its ability to discriminate patients with or without complications was limited.

KEYWORDS

post-operative outcomes, cardiopulmonary exercise, peak oxygen consumption (peak VO2), thoracoscopy (VATS), lung cancer, lung resection

Introduction

Lung cancer is currently the leading cause of death by cancer (Cancer IAfRo, 2019). Although surgery still offers the best chance to cure early stage non-small-cell lung cancer (Howington et al., 2013), pre-operative assessment and stratification of lung resection candidates is a crucial step to minimize surgical risks as this population is frequently older and comorbid (Sekine et al., 2002; Kravchenko et al., 2015).

Cardiopulmonary exercise testing (CPET) has played a central role in the evaluation of fitness of lung resection candidates. This is particularly true in patients with impaired lung function in whom various peak oxygen uptake (peak $\dot{V}O_2$) thresholds have been shown to predict increased mortality and morbidity risk following lung resection (Bolliger et al., 1995; Brutsche et al., 2000; Win et al., 2005; Loewen et al., 2007; Brunelli et al., 2009a; Fang et al., 2014; Rodrigues et al., 2016). Peak $\dot{V}O_2$ thresholds have thus been incorporated into clinical algorithms that stratify patients into low, moderate, and high risk of poor post-operative outcomes (Brunelli et al., 2013).

An important issue with this practice is that peak $\dot{V}O_2$ thresholds to predict post-operative mortality and morbidity were derived from patients who underwent thoracotomy. Video-assisted thoracic surgery (VATS) has now become widely available and the recommended surgical approach for the majority of lung resections as it is associated with less complications compared to thoracotomy (Paul et al., 2013; Laursen et al., 2016). Whether peak $\dot{V}O_2$ or any other CPET variables would still predict post-operative morbidity in this era where minimally invasive surgical procedures and improved anesthetic techniques have been implemented is uncertain.

The aim of this study was to assess if clinical and physiological variables, including peak $\dot{V}O_2$, predict post-operative mortality and cardiopulmonary morbidity in patients undergoing VATS lung cancer resection. Considering the minimally invasive nature of the surgical approach in the context of improved peri-operative care, our hypothesis was that CPET variables would not be highly predictive of post-operative outcomes in this specific population.

Methods

Subjects and study design

This is a retrospective analysis of a prospectively collected research database of all patients with a resectable lung cancer who performed CPET as part of their preoperative medical evaluation at the *Institut universitaire de cardiologie et de pneumologie de Québec* (IUCPQ), Québec, Canada. Patients who underwent VATS lung cancer surgery from 1 January 2002 to 31 December 2019 were included on a first occurrence of a non-small-cell lung cancer without prior thoracic surgery or radiotherapy and if they had pulmonary function testing and CPET data available. Surgeries were performed by thoracic surgeons at the IUCPQ where VATS progressively became the preferred approach. In 2005, VATS represented 25% of all lung cancer resections compared to 88% in 2019. CPET was also requested by the surgeons based on their clinical judgment that further testing was necessary to complete surgical risk assessment. When lung resection was considered as a potential therapeutic option, the following situations would generally trigger a CPET: forced expiratory volume in 1 s (FEV_1) < 80% predicted, age > 65 years, the presence of one or more comorbidities, and the possibility of lung resection more extensive than a lobectomy. All study participants provided informed consent and gave written approval for the use of their clinical data in subsequent research publication (CER 21184).

The primary outcome of the study was cardiopulmonary complications occurring during the 30-day period following the surgery. Baseline characteristics, pulmonary function tests, CPET, and 30-days post-operative cardiopulmonary complications and mortality were retrieved from the research database and review of the medical chart, when necessary. Age, sex, body mass index (BMI), smoking history, and extent of resection (pneumonectomy, bilobectomy, lobectomy, sublobar resection) were noted. The following comorbid conditions were documented: coronary artery disease, peripheral artery disease, hypertension, diabetes, chronic renal failure, and chronic obstructive pulmonary disease (COPD). COPD diagnosis was based on the presence of symptoms related to COPD, spirometry showing a post-bronchodilator FEV_1 to forced vital capacity (FVC) ratio < 0.70, and a smoking history of at least 10 pack-years (Vogelmeier et al., 2017). The Thoracic Revised Cardiac Risk Index (ThRCRI) was calculated for each patient (Brunelli et al., 2010). This index provides a summary score that stratifies patients into four classes, from A to D, with a progressively higher risk of post-operative cardiovascular complications.

Pulmonary function tests, including spirometry, lung volumes, and carbon monoxide diffusion capacity were performed according to previous guidelines and related to predicted normal values (Macintyre et al., 2005; Miller et al., 2005; Wanger et al., 2005). CPET results were reviewed and then adjudicated by a second author (GC, AL, and PR). Patients with CPET judged to be non-maximal according to the American College of Sports Medicine statement on CPET (Swain et al., 2000) were excluded. Briefly, when CPET was stopped before the

predicted peak $\dot{V}O_2$ was achieved, the test was considered non-maximal when maximal heart rate was not within 10 beats of the estimated maximum heart rate, minute ventilation ($\dot{V}E$)/maximum voluntary ventilation was < 85%, respiratory exchange ratio was < 1.10, and perceived exertion did not reach 7 on a 0–10 Borg scale.

Grade II or more post-operative cardiopulmonary complications on the Clavien-Dindo (Dindo et al., 2004) classification scheme (those requiring specific interventions beyond antipyretics, antiemetic, and analgesic) were recorded and definitions were based on the Society of Thoracic Surgeons and European Society of Thoracic Surgeons joint statement (Dindo et al., 2004; Fernandez et al., 2015). The following cardiopulmonary complications were recorded: need for mechanical ventilation, pneumonia, acute respiratory distress syndrome (ARDS), atelectasis, acute coronary syndrome, arrythmia, heart failure, venous thromboembolism, stroke, and acute renal failure.

Cardiopulmonary exercise testing

CPET consisted in an incremental cycling exercise performed on a cycle ergometer, until exhaustion and were performed at the clinical exercise laboratory of our institution. After 1 minute of resting on the cycle ergometer, work rate was increased in a ramp protocol using of 10- to 20-W per minute increments, with the objective of having an exercise duration of 8–10 min based on predicted peak work rate. $\dot{V}O_2$, carbon dioxide excretion ($\dot{V}CO_2$) and $\dot{V}E$ were monitored by a commercial breath-by-breath exercise circuit. Heart rate (HR), and O_2 pulse saturation (SpO_2) were continuously monitored through a 12-lead electrocardiogram and a pulse oximeter, respectively. Peak workrate and peak $\dot{V}O_2$ were expressed in abolute units and in % predicted values according to the following formulas developed by Jones and Carol (1988): Peak workrate = (25.26*height—9.08*age—2,759)*0.16344; Peak $\dot{V}O_2$ = 0.0541*height—0.025*age - 5.66; in women: Peak workrate = (12.66*height—8.27*age—940)*0.16344; Peak $\dot{V}O_2$ = 0.0301*height—0.017*age—2.56, where workrate is expressed in watts, height in cm, and age in years. Predicted peak heart rate was calculated as 210–0.65 X age in years and maximal voluntary ventilation was calculated as FEV_1 X 35 (Jones and Carol, 1988).

Statistical analysis

Results are reported as mean ± SD for continuous variables and number or % for nominal variables. Between-group comparisons were performed using the Student's t-test for continuous variables and Chi-Square or Fisher's exact test for nominal variables. We conducted univariable logistic regression analyses using potential predictors of post-surgical outcomes (age, sex, comorbid conditions, ThRCRI class C and D, extent of lung resection, FEV_1% predicted, FVC % predicted, FEV_1/FVC, total lung capacity % predicted, residual volume % predicted, carbon monoxide diffusion capacity [DLCO] % predicted, peak values for $\dot{V}O_2$ expressed in $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ and in % predicted, $\dot{V}E$, $\dot{V}E/\dot{V}CO_2$, heart rate, SpO_2) and a composite of cardiopulmonary morbidity and mortality as independent variables. Dependant variables that were associated with post-operative outcome with a $p < 0.20$ in the univariable analysis were then incorporated in a multivariable logistic regression model. Receiver operating characteristic (ROC) curve analysis was used to determine the ability of peak $\dot{V}O_2$ expressed in $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ to discriminate between patients who did nor did not experience post-operative complications. This was done by measuring the area under the ROC curve and the value at which the Youden's index (true positive rate + true negative rate -1) is maximal. An area under the ROC curve of 0.5 indicates no discrimination while values ranging from 0.7 to 0.8, 0.8 to 0.9, and > 0.9 are considered acceptable, excellent, and outstanding, respectively (Mandrekar, 2010). To determine the ability of peak $\dot{V}O_2$ expressed in $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ to predict specific types of post-operative complications, multivariable logistic models and ROC curve analyses were also conducted for: 1) pulmonary complications only, 2) cardiac complications only, and 3) grade III or more post-operative cardiopulmonary complications on the Clavien-Dindo classification scheme (Dindo et al., 2004) (those requiring surgical, endoscopic, or radiological interventions) only.

Results

Baseline characteristics of the study participants are summarized in Table 1. From 1 January 2002 to 31 December 2019, 2,228 patients underwent lung cancer resection by VATS at our institution. The study population included the 593 patients who performed a CPET pre-operatively, representing 27% of the entire VATS population. The study population included 264 men (44.5%), mean age was 66.8 years and BMI averaged 26.9 kg/m^2. COPD, hypertension, coronary artery disease, and diabetes were the most frequent comorbid conditions. Mean peak work rate was 91.4% of predicted and mean peak $\dot{V}O_2$ was 18.8 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ with a range of 7.0–36.4 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$, including four patients with a peak $\dot{V}O_2$ < 10 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$.

The overall distribution of postoperative cardiopulmonary complications according to the extent of lung resection is summarized in Table 2. Overall, 92 (15.5%) patients experienced one or more of the 130 cardiopulmonary complications within 30 days of the operation, including three deaths (0.5%), two from acute respiratory distress syndrome complicating a bronchopleural fistula and one from an acute exacerbation of pulmonary fibrosis. The most frequent complications were pneumonia (5.9%) and arrhythmias

TABLE 1 Demographic, surgical, spirometric and cardiopulmonary exercise test data (n = 593).

	Population	Without Cardiopulmonary complication	With Cardiopulmonary complication	p Value
	n = 593	n = 501	n = 92	
Demography				
Male, sex, n (%)	264 (44.5)	222 (44.3)	42 (45.7)	0.820
Age, years	66.8 ± 7.4	66.5 ± 7.6	68.5 ± 6.2	0.007
Body mass index, kg/m^2	26.9 ± 5.6	27.2 ± 5.6	26.3 ± 5.6	0.095
CAD, n (%)	114 (19.3)	93 (18.6)	21 (22.8)	0.388
PAD, n (%)	98 (16.5)	76 (15.2)	22 (23.9)	0.047
Hypertension, n (%)	318 (53.6)	259 (51.7)	59 (64.1)	0.031
Diabetes, n (%)	110 (18.6)	88 (17.6)	22 (23.9)	0.188
COPD, n (%)	330 (55.7)	273 (54.5)	57 (62.0)	0.210
eGFR, ml/min/1.73 m (Sekine et al., 2002)	80.3 ± 16.8	80.2 ± 16.6	80.6 ± 17.9	0.728
ThRCRI ≥ 2, n (%)	38 (6.4)	26 (5.2)	12 (13.0)	0.009
Surgery				
Pneumonectomy, n (%)	20 (3.4)	11 (2.2)	9 (9.8)	0.002
Bilobectomy, n (%)	31 (5.2)	23 (4.6)	8 (8.7)	
Lobectomy, n (%)	440 (74.2)	378 (75.5)	62 (67.4)	
Sublobar resection, n (%)	102 (17.2)	89 (17.8)	13 (14.1)	
Pulmonary function tests				
FEV_1, L	2.0 ± 0.6	2.0 ± 0.6	1.9 ± 0.6	0.007
FEV_1, % predicted	80.0 ± 19.0	80.9 ± 19.2	74.7 ± 16.7	0.002
FVC, L	3.1 ± 0.9	3.1 ± 0.9	2.9 ± 0.9	0.054
FVC, % predicted	94.6 ± 16.5	95.4 ± 16.8	90.3 ± 14.0	0.002
FEV_1/FVC, %	65.5 ± 11.3	65.8 ± 11.3	63.8 ± 11.3	0.111
D_LCO, % predicted	79.2 ± 23.3	80.0 ± 23.4	74.8 ± 22.0	0.049
RV, % predicted	140.3 ± 40.0	140.2 ± 40.6	140.6 ± 36.9	0.938
TLC, % predicted	108.6 ± 15.9	109.2 ± 16.1	105.9 ± 14.9	0.070
Cardiopulmonary exercise test				
Peak workrate, watts	100.7 ± 33.5	101.9 ± 33.8	94.3 ± 30.8	0.034
Peak workrate, % predicted	91.4 ± 21.6	92.2 ± 22.0	87.1 ± 18.8	0.021
Peak $\dot{V}O_2$, ml·kg^{-1}·min^{-1}	18.8 ± 4.4	19.1 ± 4.5	17.3 ± 3.9	<0.001
Peak $\dot{V}O_2$, % predicted	100.6 ± 28.3	101.7 ± 28.6	94.5 ± 26.0	0.017
Peak $\dot{V}E$, L	59.3 ± 17.5	59.9 ± 17.8	56.1 ± 15.2	0.032
Peak $\dot{V}E$/MVV	90.9 ± 24.0	90.4 ± 24.4	93.7 ± 21.6	0.143
Peak $\dot{V}E$/ $\dot{V}CO_2$	36.7 ± 7.2	36.3 ± 7.1	38.8 ± 7.5	0.003
SpO_2 at rest	97.0 ± 1.9	97.0 ± 1.9	96.8 ± 2.1	0.427
SpO_2 at peak $\dot{V}O_2$	95.8 ± 3.0	95.8 ± 3.1	95.7 ± 2.6	0.465
Peak heart rate, bpm	138.4 ± 19.6	139.1 ± 19.7	134.2 ± 18.4	0.022
Heart rate reserve, bpm	28.2 ± 18.6	27.7 ± 18.6	31.3 ± 18.8	0.096
Peak heart rate, % max predicted	83.0 ± 11.2	83.4 ± 11.2	81.2 ± 11.2	0.086

Definitions of abbreviations: CAD = coronary arterial disease; PAD = peripheral arterial disease; COPD = chronic obstructive pulmonary disease (based on Global Initiative for Chronic Obstructive lung disease - GOLD); eGFR = estimated glomerular filtration rate; ThRCRI = Thoracic Revised Cardiac Risk Index; from 0 to 5.5, based on history of ischemic heart disease, history of cerebrovascular disease, serum creatinine above 176 μmol/L (2 mg/dl), and undergoing a pneumonectomy. FEV_1 = forced expiratory volume in 1 s; FVC = forced vital capacity; D_LCO = carbon monoxide diffusion capacity; RV = residual volume; TLC = total lung capacity; Peak $\dot{V}O_2$ = oxygen uptake at peak exercise; Peak $\dot{V}E$ = ventilation at peak exercise; MVV = maximum voluntary ventilation; $\dot{V}CO_2$ = carbon dioxide excretion at peak exercise; SpO_2 = O_2 pulse saturation.

(6.8%). A larger proportion of patients who underwent bilobectomy and pneumonectomy experienced post-operative complications compared to those with less extensive resection.

Comparisons between patients with at least one post-operative cardiopulmonary complications and those who experienced an uneventful surgery are provided in Table 1. Age, peripheral artery

TABLE 2 Number of post-operative cardiopulmonary complications within 30 days of surgery according to the extent of lung resection (n = 593).

Complications	All surgery	Bilobectomy or pneumonectomy	Lobectomy	Sublobar resection	p
	n = 593	n = 51	n = 440	n = 102	
Pulmonary complications					
Mechanical ventilation	5 (0.8)	2 (3.9)	2 (0.5)	1 (1.0)	0.041
Pneumonia	35 (5.9)	5 (9.8)	24 (5.5)	6 (5.9)	0.437
ARDS	3 (0.5)	2 (3.9)	1 (0.2)	0 (0)	0.033
Atelectasis	6 (1.0)	1 (2.0)	5 (1.1)	0 (0)	0.331
Total pulmonary complications	49 (8.3)	10 (19.6)	32 (7.3)	7 (6.9)	0.154
Cardiac complications					
Acute coronary syndrome	2 (0.3)	2 (3.9)	0 (0)	0 (0)	0.007
Arrhythmia	40 (6.7)	7 (13.7)	29 (6.6)	4 (3.9)	0.090
Acute heart failure	13 (2.2)	3 (5.9)	7 (1.6)	3 (2.9)	0.070
Venous thromboembolism	4 (0.7)	0 (0)	3 (0.7)	1 (1.0)	0.698
Other	19 (3.2)	4 (7.8)	11 (2.5)	4 (3.9)	0.103
Total cardiac complications	78 (13.2)	16 (31.4)	50 (11.4)	12 (11.8)	0.026
Death	3 (0.5)	2 (3.9)	1 (0.2)	0 (0)	0.033
Total	130 (21.9)	28 (54.9)	83 (18.9)	19 (18.6)	0.003

Values are n (%). A patient may have experienced more than one cardiopulmonary complication. Abbreviation: ARDS: acute respiratory distress syndrome.

TABLE 3 Best predictors of post-operative complications based on the multivariable regression model.

Parameters	OR	95% CI
Peripheral artery disease	1.90	1.08 to 3.37
Bilobectomy (vs. sublobar)	3.44	1.20 to 9.85
Pneumonectomy (vs. sublobar)	10.32	3.29 to 32.35
Preoperative FEV_1 (% predicted)	0.98	0.97 to 1.00
Peak $\dot{V}O_2$ ($ml{\cdot}kg^{-1}{\cdot}min^{-1}$)	0.92	0.86 to 0.98
Peak $\dot{V}E/\dot{V}CO_2$	1.04	1.01 to 1.07

Definitions of abbreviations: OR = odds ratio; CI = confidence interval; FEV_1 = maximal forced expiratory volume in 1 s; Peak $\dot{V}O_2$ = oxygen uptake at peak exercise; Peak $\dot{V}E$ = ventilation at peak exercise; $\dot{V}CO_2$ = carbon dioxide excretion at peak exercise.

disease, hypertension, ThRCRI ≥ 2 (class C or D), and bilobectomy or pneumonectomy were associated with complications. FEV_1, FVC and DLCO were lower in patients with complications. Peak $\dot{V}O_2$ was 101.7% predicted in patients with an uneventful post-operative period compared to 94.5% in those with complications. This translated in a 1.8 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ difference in mean absolute peak $\dot{V}O_2$ (19.1 ± 4.5 versus 17.3 ± 3.9 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$). Consistent with these results, peak work capacity was greater in patients whose surgery was uneventful, averaging 92.2% predicted versus 87.1%. Lower peak $\dot{V}E$ and peak heart rate and higher peak $\dot{V}E/\dot{V}CO_2$ were finally noted in patients with complications.

As reported in Table 3, the best predictors of post-operative mortality and morbidity in the multivariable analysis were peripheral artery disease (odds ratio [OR] = 1.90, 95% CI: 1.08–3.37), bilobectomy (OR = 3.44, 95%CI: 1.20 to 9.85 versus sublobar resection), pneumonectomy (OR = 10.32, 95%CI 3.29 to 32.35 versus sublobar resection), FEV_1% predicted (OR = 0.98 per unit, 95%CI: 0.97–1.0), peak $\dot{V}O_2$, $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ (OR = 0.92 per unit, 95%CI: 0.86–0.98), and peak $\dot{V}E/\dot{V}CO_2$ (OR = 1.04 per unit, 95%CI: 1.01–1.07).

To further explore possible relationships between peak $\dot{V}O_2$ and adverse post-operative outcomes, the proportion of patients experiencing at least one cardiovascular complication was documented according to categories of baseline peak $\dot{V}O_2$ $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ (table 4). Only four patients (0.7%) had a peak $\dot{V}O_2$ < 10 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$. Peak $\dot{V}O_2$ between 10 and < 15 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$, 15 to < 20 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$, and ≥ 20 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ was found in 101 (17%), 276 (47%), and 212 (36%) individuals, respectively. The proportion of patients experiencing complications in these peak $\dot{V}O_2$ categories was 24.8, 16.3, and 10.4%, respectively (p = 0.01 between groups).

The ROC curve for peak $\dot{V}O_2$ $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ to discriminate for the occurrence of cardiopulmonary post-operative complications in provided in Figure 1. The area under the ROC curve for peak $\dot{V}O_2$ was 0.63 (95% CI: 0.57–0.69) and the maximum value of the Youden's index was 0.22 at a peak $\dot{V}O_2$ of 17 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$. At this peak $\dot{V}O_2$, the true positive rate i.e. the sensitivity (the proportion of patients who experienced a post-operative complication who had a peak $\dot{V}O_2$ < 17 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) was 56.5% and the false positive rate i.e., 100 – specificity % (the proportion of patients who did

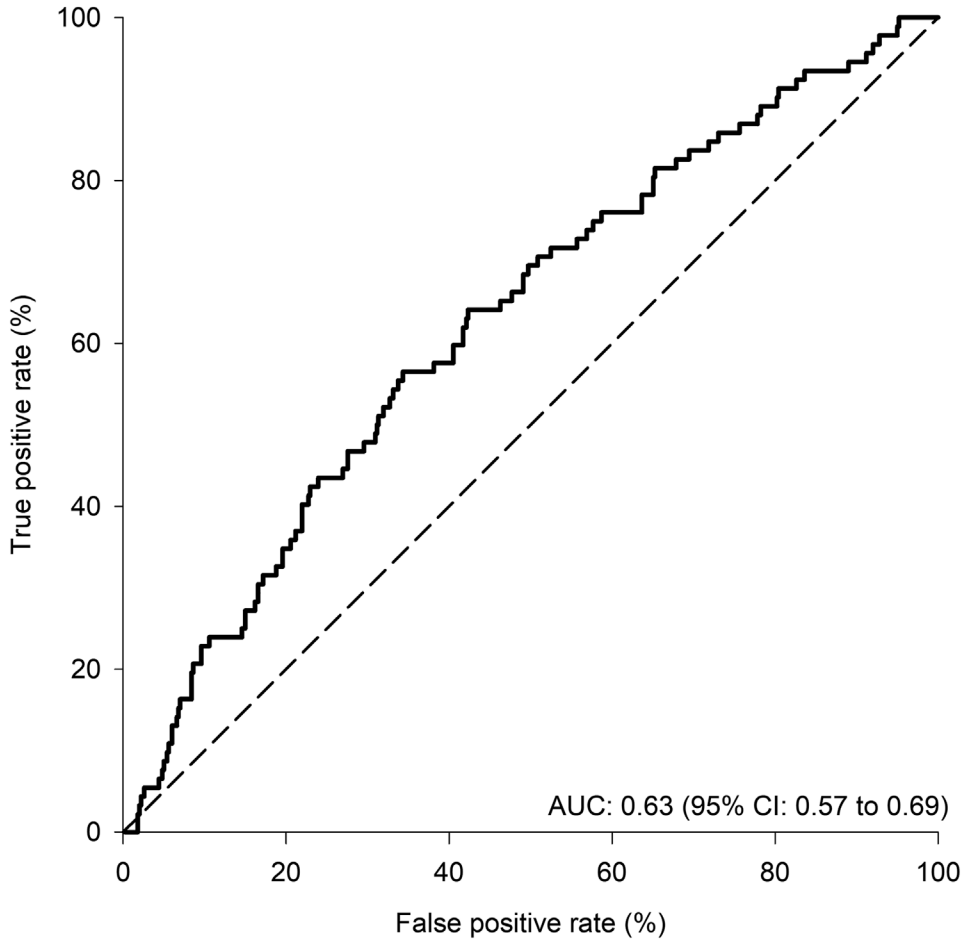

FIGURE 1
Receiver operating characteristic (ROC) curve for peak $\dot{V}O_2$ expressed in ml·kg^{-1}·min^{-1}. The dash line indicates a situation of a test that would have no discriminatory value for the occurrence of post-operative complications, with an area under the curve (AUC) of 0.5.

not experience a post-operative complication who had a peak $\dot{V}O_2$ < 17 ml·kg^{-1}·min^{-1}) was 34.3%.

The ability of peak $\dot{V}O_2$ ml·kg^{-1}·min^{-1} to predict post-operative complications was not improved by restricting the analysis to specific complications and the results were consistent across various types of complications. The ORs of experiencing only pulmonary, only cardiac, or only grade III or more complications varied from 0.90 to 0.95 per unit of increase in peak $\dot{V}O_2$, with area under the ROC curve varying from 0.56 to 0.63, and the best discriminative value for peak $\dot{V}O_2$ ml·kg^{-1}·min^{-1} ranging between 17 and 18 ml·kg^{-1}·min^{-1} for these specific complications. We also calculated the ability of peak $\dot{V}O_2$ ml·kg^{-1}·min^{-1} to predict post-operative complications according to baseline FEV_1 (≥ 80% predicted and < 80% predicted). We found that the discriminative ability of peak $\dot{V}O_2$ ml·kg^{-1}·min^{-1} was similar between the two FEV_1 categories with ORs of experiencing post-operative complications of 0.89 (0.81–0.97) and 0.93 (0.86–1.00) per unit of increase in peak $\dot{V}O_2$ in the former and latter group, respectively. The corresponding area under the ROC curve was 0.64 and 0.59.

Discussion

Based on the consistent observation that peak $\dot{V}O_2$ during CPET is inversely related to the risk of mortality and morbidity following lung resection (Benzo et al., 2007), the use of CPET has been endorsed by European (Brunelli et al., 2009b) and American (Brunelli et al., 2013) guidelines for risk stratification in lung cancer resection candidates. Data supporting these recommandations were obtained in patients whose lung resection was peformed by thoracotomy (Bolliger et al., 1995; Brutsche et al., 2000; Win et al., 2005; Brunelli et al., 2009a). The present study explored whether these early findings also apply to minimally invasive lung resection performed by VATS. Despite the observation that lower peak $\dot{V}O_2$ was associated with a higher

likelihood of cardiopulmonary complications after VATS lung cancer resection, the ability of peak $\dot{V}O_2$ to predict post-operative cardiopulmonary complications in individual patients was limited. This was true for a composite of cardiopulmonary morbidity and mortality as well as for respiratory and cardiac complications, separately and for the more severe complications (grade III or more).

The past 20 years has witnessed a consistent trend in better post-operative outcome after lung cancer resection, with 30-day post-operative mortality decreasing from 3–5% to 1% (Morgant et al., 2015; Shewale et al., 2020). Improved outcome following lung resection is related to the use of minimally-invasive operative techniques that are associated with less complications than thoracotomy (Boffa et al., 2014; Laursen et al., 2016), but also to improved patient selection, anesthetic techniques, and general postoperative medical management (Morgant et al., 2015; Shewale et al., 2020). Although it is difficult to compare post-operative outcome across studies due to differences in patient populations and in how post-operative complications are documented and defined, our results are consistent with the published experience with VATS lung cancer resection (Boffa et al., 2014; Begum et al., 2016; Shewale et al., 2020).

Considering the lower complication rates that is now observed after lung resection, a lower discriminative ability could be expected from preoperative evaluating tests. This has been suggested by Berry *et al* who reported that pulmonary function tests lose their predictive ability for pulmonary complications in patients who underwent thoracoscopy for lung resection (Berry et al., 2010). Nonetheless, our results show that despite relatively low mortality and morbidity rates of 0.5 and 15.5%, respectively, peak $\dot{V}O_2$ was still inversely related to the occurrence of postoperative outcomes. Indeed, peak $\dot{V}O_2$ emerged as an independent predictor of mortality and morbidity in the multivariable analysis and there was a significant trend in lower complication rates with progressively higher peak $\dot{V}O_2$ as indicated by a prevalence rate of 24.8, 16.3, and 10.4% for peak $\dot{V}O_2$ of <15, 15 to <20, and ≥20 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$, respectively. Despite this, the ability of peak $\dot{V}O_2$ to discriminate between patients who experienced or not an adverse outcome was limited. The area under the ROC curve for peak $\dot{V}O_2$ was only 0.63 (95% CI: 0.57–0.69), below the 0.7 threshold that would be considered acceptable for a diagnostic tool (Mandrekar, 2010). Thus, according to the area under the ROC curve, the odds of misclassifying a patient for the occurrence of post-operative cardiopulmonary complications would be 37% ([1—area under the curve]*100). Further, the proportion of patients who experienced a post-operative complication and who had a peak $\dot{V}O_2$ < 17 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ (true positive rate) was 56.5% and the proportion of patients who did not experience a post-operative complication and who had a peak $\dot{V}O_2$ < 17 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ (false positive rate) was 34.3%. The role of CPET in risk stratifying for VATS was also questioned by Begum *et al* who reported similar postoperative morbidity and mortality after VATS lobectomy for patients with high (≥ 15 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) versus low (< 15 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) peak $\dot{V}O_2$ (Begum et al., 2016).

Besides peak $\dot{V}O_2$, numerous CPET findings were associated with complication rates in the present cohort. Lower peak work rate, peak $\dot{V}E$ and peak heart rate, and higher peak $\dot{V}E/\dot{V}CO_2$ were all associated with higher complication rate, but only the latter remained significantly associated with post-operative outcome in the multivariable analysis. This parameter had previously been described as a good predictor of postoperative complications in thoracotomy patients (Torchio et al., 2010; Brunelli et al., 2012). Although this finding makes sense from a physiological perspective, the small difference noted between patients with and those without complications makes peak $\dot{V}E/\dot{V}CO_2$ difficult to apply to predict of postoperative outcomes.

Strengths of the current study include its large sample size of patients who underwent VATS lung resection and in whom comprehensive CPET data were available. All exercise tests were carried out in the same clinical exercise laboratory and their validity was confirmed by three investigators. We acknowledge that our study has several limitations including its retrospective nature. To minimize the possibility of under reporting complications, we relied on a research database where clinical data is entered prospectively, and this was complemented by the review of medical charts, when necessary. Because only four patients with peak $\dot{V}O_2$ below 10 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ were included in this cohort, our results do not necessarily apply to patients with very poor exercise tolerance. The retrospective nature of our study did not allow to retrieve nadir $\dot{V}E/\dot{V}CO_2$ values which could have been more informative to predict the occurrence of post-operative complications that $\dot{V}E/\dot{V}CO_2$ at peak exercise. By design, only patients who had surgery were considered for this study and we cannot exclude that CPET results may have led to recommending medical treatment in some patients with poor CPET performance. Nevertheless, 105 patients with a peak $\dot{V}O_2$ < 15 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ that would classify them as intermediate risk according to current guidelines (Brunelli et al., 2009b; Brunelli et al., 2013) were included, supporting the external validity of the results. Another limitation is the study sample which represents only 27% of the total VATS lung resection population in our institution. The present study represents a real-life situation where the decision to order a CPET pre-operatively was left at the discretion of the surgeons according to their clinical judgment regarding risk categorization, without the use of standardized protocol which would have been preferable from a methodological standpoint considering that CPET could be more discriminative in certain subsets of patients that remain to be identified. It is nevertheless informative to note that in the present study, peak $\dot{V}O_2$ performed similarly in patients with FEV_1 < and ≥ 80% predicted. Lastly, the generalizability of our data is uncertain given that we are reporting a single centre experience.

Conclusion

Although peak $\dot{V}O_2$ was associated with postoperative outcomes in this cohort of patients who underwent lung cancer VATS lung resection, its ability to discriminate for the occurrence of post-operative cardiopulmonary complications was limited. Based on these results, patients should not be denied lung resection on the basis of peak $\dot{V}O_2$ when minimally invasive resection is feasible. Prospective studies incorporating CPET results in the preoperative algorithm are needed to better define the place of peak $\dot{V}O_2$ in the evaluation of patients undergoing VATS resection for lung cancer.

Data availability statement

The raw data supporting the conclusions of this article will be made available anonymously on request without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Comité d'éthique de l'Institut universitaire de cardiologie et de pneumologie de Québec. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GC, PR, AL, EM, MM, YL, and FM conceived and designed the work. All authors were involved in data acquisition. GC, PR, AL, EM, YL, and FM analyzed the data and drafted the work. All authors contributed to critical reading of the drafted manuscript, to the completion of the final document, and to its approval. GC, PR, YL, and FM are accountable for all aspects of the work and to the accuracy and integrity of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The validity and reliability of counter movement jump height measured with the Polar Vantage V2 sports watch

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The purpose of the present study was to assess the validity and reliability of the jump height measured by the Polar Vantage V2 sports watch in comparison to a gold-standard force plate measurement. Fifteen healthy adults, seven female, age 20–42 years participated in the study and performed six sets of three CMJs, on two consecutive days. The participants wore the Polar Vantage V2 sports watch (Polar Electro Oy, Kempele, Finland) whilst performing the jumps on two force plates (AMTI, Watertown, Massachusetts, United States). Jump height was on the one hand extracted directly from the watch ("leg recovery test") and on the other hand calculated by the flight time method with the force plate data. To assess validity, we calculated the mean absolute error, constructed Bland-Altman plots and applied an ordinary least squares regression analysis. To test for left-to-right and day-to-day reliability, we calculated Pearson and intraclass correlations. We found a mean error of ≈5% and a high correlation ($r = 0.96$; $p < 0.001$) for the jump height measured by the Polar Vantage V2 sports watch compared to the force plate measurement. The Bland-Altmann plot together with the ordinary least squares regression analysis showed no systematic bias between the methods with a minimal difference at a jump height of 30 cm. For reliability of left-to-right and day-to-day measurements, we found high Pearson and ICC correlations and no indications for systematic bias by Bland-Altmann analysis. The present study has demonstrated that the "leg recovery test" of the Polar Vantage V2 sports watch provide a valid and reliable measurement of the mean vertical jump height of three consecutive CMJs. For the first time the jump height of a CMJ can be measured solely by a sports watch without the need to attach additional sensors or measurement devices. Thus, the "leg recovery test" is an easy to administer, valid and reliable test, that can be used in future studies to measure CMJ-height in the field when lab-based assessments are unavailable or inconvenient. This opens new avenues for cross-sectional and longitudinal assessments of neuromuscular power of the lower extremities in a large number of participants.

KEYWORDS

vertical jump, IMU, flight time, neuromuscular power, leg extension, performance, fitness test

Introduction

The countermovement jump (CMJ), which can be considered as the natural way for human beings to jump as high as possible without a run-up, has been extensively used to test neuromuscular performance in humans (for review see (1)). As jump height is a direct result of the generated impulse during leg extension, CMJ height is not only a task-specific measure of performance but reflects well the overall functional status of an individual (1). This is underlined by the fact that peak power during a CMJ is normally produced with jumping at bodyweight and neither loading nor unloading the body can increase peak power during the movement substantially (2). This optimum loading principle emphasize the functional importance of the CMJ as a test able to determine neuromuscular performance for daily life activities, like running, walking stairs, or simply standing up from a chair or bed (3). In this regard, CMJs have been successfully used to cross-sectionally assess neuromuscular performance in children (4), young adults (5) and elderly (6) and are further expected to be an effective type of exercise for sedentary people (7) as well as to monitor longitudinal effects of exercise [for review see (8)].

The gold standard to measure different outcome parameters of CMJs is a force plate measurement. As all forces that act at the bodies center of mass during the jump can be measured *via* the ground-reaction force, the jump height can easily be calculated from the force data. Jump height can be calculated *via* the flight time (time of zero force) with the classical law of ballistics, or it can be calculated by integration of the measured force over time during the jump (impulse method). Both methods have been shown to be valid (9), however, special care should be taken when directly comparing jump heights measured by the two distinct methods. Usually, the initial position for participants during the test is an upright stance with the entire foot on the ground. When jumping from this position the center of mass displacement derived by the impulse method is the difference of the center of mass during flat stance and the maximum height during the jump whereas for the flight time method the flight phase starts obviously at take-off position with a more extended ankle joint. Thus, jump heights that are calculated by the flight time method are obviously lower when directly compared to the impulse method (10).

For obvious reasons the calculation of jump height by impulse is restricted to force plate measurements whereas flight time can be detected by simpler measurement techniques. Recently, sensor-technology developed rapidly and a variety of systems have been brought to market maturity and have also been validated scientifically (for review see (11)). In principle two different categories of measurement methods can be distinguished. The first category comprises devices that measure the contact to the ground, like contact mats (12), light-barrier-systems (13) or video systems (14). The second category comprises inertial measurement units (IMUs) which are usually composed of accelerometers to detect linear acceleration and gyroscopes to measure rotational rate (see Table 4). Whereas in the first category the contact of the feet to the ground is measured with a device that is placed stationary outside the moving body (contact mat, light-barrier-system, camera), IMUs are attached to the body to capture the jump directly. In this regard IMUs provide the most feasible way to measure jump height outside the lab and in a large number of people. This is of utmost importance to obtain benefit from the CMJ-test in population studies and in broadly disseminating the test for the assessment of neuromuscular performance by practitioners (15).

The advantage of the measuring device being attached to the participants' body comes with the disadvantage that the attachment site of the IMU has to be chosen carefully. To the best of our knowledge only IMUs attached to the foot or upper/lower back (see Table 4) have been validated so far. The reported biases, random errors and correlations with jump heights calculated *via* force plate data in these studies (14, 16–20) show that IMUs in principle can be used to measure the jump height during a CMJ. Recently, Polar© integrated the "leg recovery test" into their Vantage V2 sports watch. The test consists of three CMJs and jump height is measured by an IMU, that is located inside the watch. To our knowledge, for the first time the jump height can be measured by a sports watch without the need to attach an additional sensor to the body. Thus, the test is technically and economically feasible to be used in a large population outside the lab in a real-world environment. The purpose of the present study was to assess the validity and reliability of the jump height measured by the Polar Vantage V2 sports watch in healthy adults.

Methods

Participants

Fifteen healthy adults (8 men and 7 women) aged 20 to 42 years (M = 26.4, ±5.6 years) took part in the study at the Human Performance Research Centre (HPRC) of the University of Konstanz. The study protocol was in accordance with the Declaration of Helsinki for human experimentation and the ethics standards of the University of Konstanz. The subjects were informed of the experimental risks and data processing procedures and signed an informed consent document prior to participation. Participants with acute severe injuries or with an injury in the last 6 months were excluded from the study (21).

Study design

The participants performed two test sessions on subsequent days. On both days they first performed a standardized warm-up consisting of 20 jumping jacks, 10 squats and 10 sub-maximal CMJs. Afterwards, they performed three sets of three CMJs on two force plates (AMTI OR-6, Watertown, Massachusetts, United States), while wearing the Polar Vantage V2 sports watch (Polar Electro Oy, Kempele, Finland) and recording the "leg recovery test" which is integrated in the sports watch. Each set was separated by a 3-mins rest period and the rest period between individual jumps was 1 min. One set was performed with the Polar Vantage V2 at the right wrist, one set with the watch at the left wrist and one set with both watches simultaneously worn at the left and the right wrist. We used two different watches for the measurements, one watch was defined in the settings as a watch for the left wrist and another one was defined as a watch for the right wrist. The following day, the same watches were used for the same sides. In between subjects the order of sets was randomized.

The Polar Vantage V2 sports watch calculated the flight time of the CMJ by identifying the time between the take-off and landing by means of analysing the IMU data and then converting it to a jump height using the equation below where h is the jump height in meters, t is the flight time of the jump in seconds and g (9.81 ms^{-2}) is the gravitational acceleration (22).

$$h = \frac{1}{2}\left(\frac{t}{2}\right)^2 g$$

The same equation was used to calculate jump heights from the force plate data using an in-house written MATLAB (Version R2022a, The MathWorks, Inc., Natick, Massachusetts, United States) script.

In addition, we calculated jump height with the impulse method using an in-house written MATLAB script. We calculated the impulse (J) as the integral of the force (F) over the time interval from standing still on the force plate (t1) to take off (t2) which includes the whole countermovement (downward and upward phases of the jump). We used the trapezoidal rule for approximating the definite integral.

$$J = \int_{t1}^{t2} F\,dt$$

Then, we calculated the take-off velocity (v) using the following formula, with m being the participant's mass:

$$v = \frac{m}{J}$$

We calculated the jump height (h) with the following formula with v as the take-off velocity and g (9.81 ms-2) the gravitational acceleration.

$$h = \frac{v^2}{2g}$$

We included this analysis as supplementary data (Supplementary Figure A, Table A).

Material

Sports watch

The Polar Vantage V2 sports watch (Polar Electro Oy, Kempele, Finland) integrates the measurement of jump height within the "leg recovery test." The sports watch is worn at the wrist and uses the data of IMUs that are placed inside the watch to calculate the jump height of a CMJ. We used the instructions given by Polar: a) to prepare for the jump by placing hands firmly on hips and standing straight, b) to squat quickly and jump as high as possible by supplying power equally from both legs, c) not to bend the knees in the air before touchdown, d) to bend the knees after touchdown to allow smooth landing, e) to keep hands on hips throughout the entire movement (23). The watch displays the jump height for each individual jump in cm and the mean of the three jumps in cm without decimal places. We extracted the mean of the three jumps (one set) from the watch and used it for further analysis.

Force plate

The force plates (AMTI OR-6, Advanced Mechanical Technology Inc., Waterton, Massachusetts, United States) recorded the data at a sampling frequency of 1,000 Hz and were used to measure the flight time of the CMJs at the same time as they were recorded on the Polar Vantage V2 sports watch. They were connected to a computer equipped with the analysis software Vicon Nexus 2.10 (Vicon Motion Systems Ltd., Yarnton, United Kingdom). We defined the flight time between take-off and landing as the time during which the force was equal or <30 N. This time was then taken to calculate the jump height using the equation as described above (22).

In addition, we calculated the impulse and derived the jump height with the impulse method (see Bland-Altman Plots Supplementary Figure A, Table A).

Statistical analysis

We processed and analyzed all data with Excel (Version 16.53), MATLAB (Version R2022a) and JASP (Version 0.14.1) and calculated mean values, standard deviation (SD) as well as

the coefficient of variation, as the ratio of the standard deviation (SD) to the mean, for all variables.

We calculated the mean absolute error (MAE) and the mean absolute percentage error (MAPE) with the following formulas, where yi is the jump height [cm] calculated by the force plate data, xi is the jump height [cm] calculated by the Polar Vantage V2 and n is the total number of measurements:

$$MAE = \frac{1}{n} * \sum_{i=1}^{n} |y_i - x_i|$$

$$MAPE = \frac{100\%}{n} * \sum_{i=1}^{n} \left| \frac{y_i - x_i}{y_i} \right|$$

TABLE 1 Descriptive statistics and comparison between the CMJ-height measured by the Polar Vantage V2 sports watch and calculated *via* the flight time method from the force plate data.

	Polar Vantage V2	Force plate
Mean [cm]	29.93	30.24
Standard deviation [cm]	6.28	6.79
CV	0.21	0.22
Pearson's *r*[95% CI]	0.96*** [0.94,0.97]	
Mean absolute error [cm]	1.54	
Mean absolute percentage error [%]	5.19	

Level of significance: ***p < 0.001.

In addition, we constructed Bland-Altman plots, to provide a representation of the agreement between the two methods (24) and applied an ordinary least squares (OLS) regression analysis to identify any systematic bias.

To test for reliability, we followed the recommendations of (25). We used Pearson's and intraclass correlation (ICC) to analyse left-to-right (jump height measured with the watch at the right wrist vs. jump height measured with the watch at the left wrist) and day-to-day reliability (jump heights measured during day 1 vs. jump heights measured during day 2). We considered Pearson's R

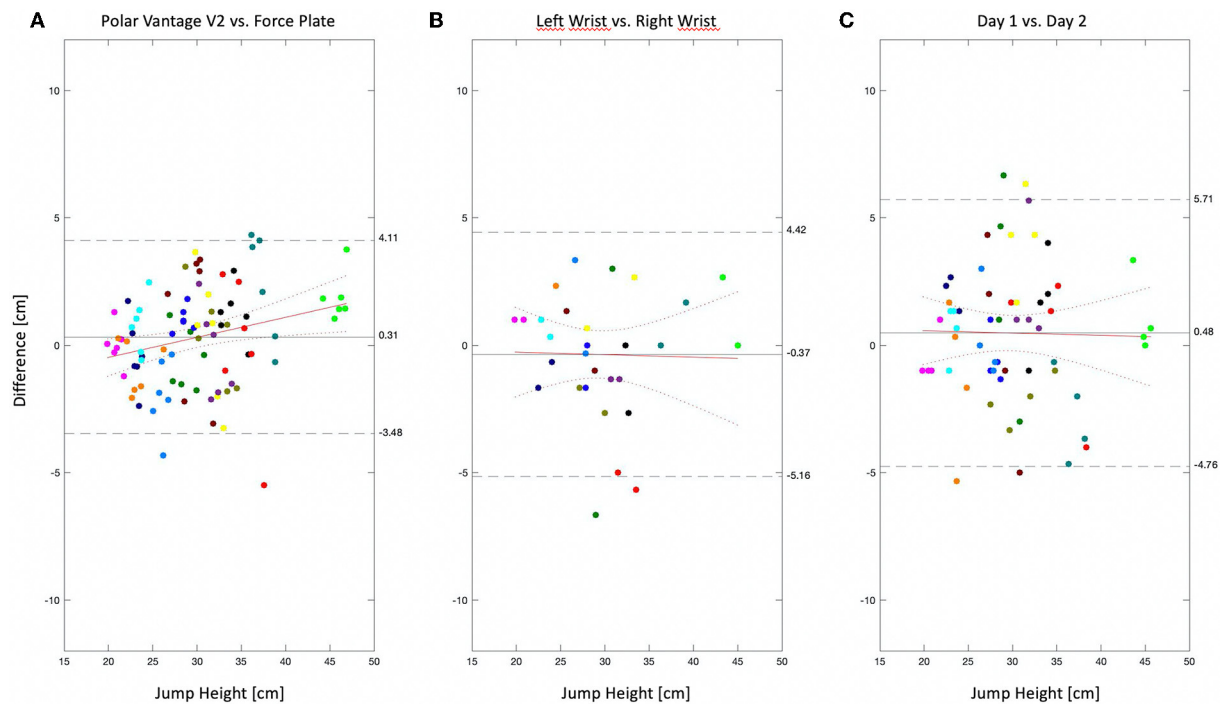

FIGURE 1
Bland-Altmann plots for **(A)** the difference of the jump height measured by the Polar Vantage V2 sports watch compared to the force plate measurement (jump height measured by force plate data—jump height measured by the Polar Vantage V2). **(B)** The left-right difference in CMJ-height when wearing one watch on the left and another watch on the right hand simultaneously (jump height measured by the Polar Vantage V2 on the left wrist – jump height measured by the Polar Vantage V2 on the right wrist) and **(C)** the day-to-day difference in CMJ-height (jump height measured by the Polar Vantage V2 on day 2 – jump height measured by the Polar Vantage V2 on day 1). The OLS regression lines are indicated as solid red lines with the 95% confidence intervals indicated as red dotted lines. The limits of agreement are marked as dashed black lines and given in numbers on the right side of the three plots together with the bias (mean of the differences) marked as solid black line. Same colored dots represent the mean of three jumps of one participant. Values are plotted over the CMJ-height derived *via* the flight time method from the force plate measurements.

scores of $r > 0.8$ (26) and ICC scores > 0.75 as high correlations (27).

Results

The mean jump heights measured by the Polar Vantage 2 sports watch and the AMTI force plates were 29.93 cm (± 6.28 cm) and 30.24 cm (± 6.79 cm) respectively (Table 1). We were able to show a high correlation ($r = 0.96$; $p < 0.001$) between both methods and found a mean absolute percentage error of 5.19% for the jump height measured by the Polar Vantage V2 sports watch compared to the jump heights calculated by the force plate measurement. Figure 1A shows the comparison between the jump heights measured with the Polar Vantage V2 sports watch and the jump height measured with the force plates. In total, bias was 0.31 cm (higher jump height measured by the Polar Vantage V2). Please note that the slope of the regression line differed significantly from zero [$F_{(90)} = 6.53$; $p = 0.01$ with an $R^2 = 0.06$] with the intersection point at 30 cm jump height, indicating that the difference between the jump heights derived from the force plate data and jump heights measured by the Polar Vantage V2 increase with jump heights that are lower or higher than 30 cm.

Results for the left-right reliability as well as for the test-retest (day-to-day) reliability are shown in Tables 2, 3. For the reliability analysis we only refer to jump heights measured by the Polar Vantage 2 sports watch. We found a high correlation between the jump heights measured while wearing the watch at the left wrist compared to wearing the watch at the right wrist respectively ($r = 0.92$, $p < 0.001$; ICC = 0.96, $p < 0.001$). For the day-to-day correlations, we found high scores ($r = 0.91$, $p < 0.001$; ICC = 0.79, $p < 0.001$) between the jump heights that were measured on consecutive days. In addition the regression analysis (Bland-Altmann Plots) showed no bias for either the left-to-right [$F_{(30)} = 0.02$; $p = 0.90$; $R^2 \leq 0.001$; Figure 1B] nor the day-to-day reliability [$F_{(60)} = 0.03$; $p = 0.88$; $R^2 \leq 0.001$; Figure 1C].

TABLE 2 Descriptive statistics and correlations of the left-right comparison between the CMJ-height measured by one Polar Vantage V2 sports watch attached to the right wrist and another one simultaneously attached to the left wrist.

	Right wrist	Left wrist
Mean [cm]	29.57	29.20
Standard Deviation [cm]	6.18	6.12
CV	0.21	0.21
Pearson's r [95% CI]	0.92*** [0.84,0.96]	
ICC [95% CI]	0.96*** [0.92,0.98]	

Level of significance: ***p < 0.001.

TABLE 3 Descriptive statistics and correlations of the day-to-day comparison between the CMJ-height measured by Polar Vantage V2 sports watch on two consecutive days.

	Day 1	Day 2
Mean [cm]	29.56	30.03
Standard deviation [cm]	6.31	6.25
CV	0.21	0.21
Pearson's r [95% CI]	0.91*** [0.85,0.95]	
ICC [95% CI]	0.79*** [0.73,0.84]	

Level of significance: ***p < 0.001.

Discussion

The most important result of the present study is a rather small bias of 0.31 cm and mean absolute percentage error of 5.19% together with high correlations between CMJ-heights measured by the Polar Vantage V2 sports watch compared to the CMJ-heights measured *via* the force plates (see Figure 1A; Table 1). Error and bias are in the same range (17, 18, 20) or even lower (16, 19) compared to other studies that evaluated various IMU measurements against force plate derived CMJ-heights. Besides similarities in outcome, the biggest difference between the Polar Vantage V2 and the other devices, however, is the sensor placement (see Table 4). Whereas the IMU of the Polar Vantage V2 is located inside the sports watch itself, the IMUs of the other devices need to be attached to the body, usually foot or trunk. This comes with the big advantage that the test application is much simpler when using the Polar Vantage V2, but it comes with the disadvantage that you need to keep hands strictly attached to the hips during the CMJ. This difference has great implications for the potential field of application. Thinking of the assessment of jump heights during daily activities and sports, like e.g., the measurement of jump heights during a soccer game, the Polar Vantage V2 sports watch is inappropriate in its present condition. The preferred area of application of the Polar Vantage V2 can clearly be seen in the assessment of CMJ-heights in a broad population where the tests have to be conducted outside a laboratory with only minor or no involvement of experts and on a regular base. In this regard testing CMJ-height with the Polar Vantage V2 sports watch can be an appropriate measure to increase sample size in studies that aim to test neuromuscular performance cross-sectionally over the life-span (4–6) or longitudinally to test training interventions (7, 8). Recently, Dijkstra et al. (15) defined the term e-Sport-and-Exercise-Medicine (eSEM) as "the practice of SEM in athlete and public health contexts supported by electronic processes and communication." Within this context a simple test like the CMJ can gain further importance for the assessment of neuromuscular performance (5, 28), which has

TABLE 4 Overview on studies that validated the measurement of CMJ-height by an IMU-device against CMJ-height calculated from force plate data.

Study	IMU type	Placement	Correlation jump height IMU vs. Force plate (ICC or Pearson's r)	Error jump height IMU vs. Force plate (Random error, Bias)
Garnacho-Castaño et al. (17)	Polar stride sensor (tri-axial accelerometer, sampling rate 100 Hz)	Sport shoe	ICC 0.97 (0.94–0.98 95% CI)	Bias−0.45 cm, Random error 1.85 cm
Choukou et al. (16)	Accelerometer (*Myotest*, sampling rate 500 Hz)	Middle of the lower back	ICC 0.79–0.86 (95% CI)	Bias 3.6 cm, random error 13.1 cm
Rantalainen et al. (19)	Accelerometer (sampling rate 100 Hz)	Back mid-line between scapulae at T1-T5 level	ICC 0.959 (95% CI)	Bias 4.3 cm
Pino-Ortega et al. (18)	WIMU (Realtrack, sampling rate 1,000 Hz)	Lower back on a belt	ICC 0.97 (0.96–0.98 95% CI)	Bias 0.29%
Setuain et al. (20)	IU (MTx, Xsens Technologies, sampling rate 100 Hz)	Middle of the lower back	Pearson's r 0.96 (0.89–0.99, $p > 0.001$)	Bias 1.96 cm

been shown to act as a risk factor for frailty and other age related diseases (29, 30).

In line with previous studies that looked at comparing new non-IMU based measurement methods for jump height acquisition with force plate measurements, like the MyJump-App (14, 31) or the G-Flight micro-laser system (32), the results of the present study showed a slight overestimation of mean jump height at jump heights >30 cm but also an underestimation of jump heights below a jump height of 30 cm. Thus, the algorithm used to calculate the jump heights with the IMU data of the Polar sports watch shows its optimal performance at a jump height of 30 cm. It can be speculated that this is a result of the optimisation of the algorithm that is integrated in the Polar Vantage V2 at mean jump heights of an average study population, like the participants of the present study with an average jump height of 30 cm (Table 1). We were not able to find similar studies investigating jump height using wearables compared to force plates, that showed the same behavior. However, studies comparing jump-mats and motion capture systems showed similarly large differences between the methods at jump heights >0.30 cm (9, 33).

It has to be noted that the jump heights calculated *via* the flight time significantly differ from the jump height calculated based on the impulse derived from the measurement of the ground reaction force during the jump (see Supplementary material). The mean jump height calculated *via* the impulse method was 36.52 cm and thus 6.28 cm higher than the jump height calculated *via* the flight time method based on the force plate data and 6.59 cm higher than the jump height calculated by the Polar Vantage V2 sports watch (see Table 2; Supplementary material). This can be explained by a different definition of jump height between the methods and is well in line with the literature (10).

In a second part of the study, we checked the left-to-right and the day-to-day reliability of the CMJ-height measured by the Polar Vantage V2 sports watch. A low bias and high correlations (see Figure 1B; Table 2) between the jump heights of the simultaneous measurement of two watches, one at the left and one at the right wrist, indicated no systematic difference. Thus, the watch can be worn at either side without violating the measurement of CMJ-height. We obtained similar results, low bias and high correlations, also for the day-to-day reliability (see Figure 1C; Table 3). The day-to-day reliability provides the necessary precondition that can make the Polar Vantage V2 sports watch a valuable tool for longitudinal studies and a potential candidate within an eSEM strategy that aims to incorporate an outcome of neuromuscular performance.

The present study has several limitations, that should be considered. First and foremost, only fifteen participants took part in our study. Each of the participants performed six sets of three jumps each, resulting in six data points per participant (see Figure 1A). For the analyses we took the six means, which are not independent measures, into calculation, harbouring the risk to overvalue the correlations. Second, it has to be noted that the limits of agreement (see Figure1A) are still−3.48 cm and +4.11 cm respectively, meaning that the jump height for one person can differ by quite a large margin. We therefore argue that although the results may be valid for the mean of a large population, the results from an individual participant should be carefully considered. Third, we only tested healthy adults. A simple generalization of the results to groups of all age (children and seniors) and performance level (athletes) can therefore not be done easily.

Conclusion

The "leg recovery test" of the Polar Vantage V2 sports watch can be used as a valid and reliable tool to assess the mean jump height of three successive CMJs. Without the need to attach any additional sensor to the body and given a good reliability over time and between sides, the sports watch provides an easy-to-perform test procedure that can be used to measure CMJ-height within a significant number of people. We suggest taking advantage of this technology to collect jump data from participants without the need for laboratory-based measurements thus enabling large-scale studies to be conducted at comparatively low costs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MG devised the project, designed the study, contributed to data analysis, and drafted the manuscript. JP contributed to the design of the study, helped with the data analysis, and reviewed the manuscript. JB helped with the experiments and data analysis and reviewed the manuscript. PB carried out the experiments, performed the computations and data analysis, and drafted parts of the manuscript. All authors contributed substantially to the article and approved the submitted version.

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Conflict of interest

JP is employed as Senior Researcher at Polar Electro Oy, Kempele.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspor.2022.1013360/full#supplementary-material

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Differences in time to task failure and fatigability between children and young adults: A systematic review and meta-analysis

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The transition from childhood to adulthood is characterized by many physiological processes impacting exercise performance. Performance fatigability and time to task failure are commonly used to capture exercise performance. This review aimed to determine the differences in fatigability and TTF between youth (including both children and adolescents) and young adults, and to evaluate the influence of exercise modalities (i.e., exercise duration and type of exercise) on these differences. Medline, SPORTDiscus and Cochrane Library were searched. Thirty-four studies were included. The meta-analyses revealed that both children (SMD –1.15; $p < 0.001$) and adolescents (SMD –1.26; $p = 0.022$) were less fatigable than adults. Additional analysis revealed that children were less fatigable during dynamic exercises (SMD –1.58; $p < 0.001$) with no differences during isometric ones (SMD –0.46; $p = 0.22$). Children (SMD 0.89; $p = 0.018$) but not adolescents (SMD 0.75; $p = 0.090$) had longer TTF than adults. Additional analyses revealed 1) that children had longer TTF for isometric (SMD 1.25; $p < 0.001$) but not dynamic exercises (SMD –0.27; $p = 0.83$), and 2) that TTF differences between children and adults were larger for short- (SMD 1.46; $p = 0.028$) than long-duration exercises (SMD 0.20; $p = 0.64$). Children have higher endurance and are less fatigable than adults. These differences are influenced by the exercise modality, suggesting distinct physiological functioning during exercise between children and adults. The low number of studies comparing these outcomes between adolescents versus children and adults prevents robust conclusions and warrants further investigations in adolescent individuals.

KEYWORDS

children, adolescents, fatigability, time to task failure, neuromuscular physiology

1 Introduction

There has been a growing interest in recent decades for the evaluation of exercise-induced fatigue in children, with numerous reports investigating potential child-adult differences regarding its magnitude and etiology (Ratel et al., 2006a; Patikas et al., 2018).

Fatigue is a multifactorial and complex concept, and a new taxonomy has been recently proposed to acknowledge its attributes that are performance and perceived fatigability (this latter also referred to as perception of fatigue). Those two attributes are closely interrelated and inseparable (Kluger et al., 2013; Enoka and Duchateau, 2016; Gruet, 2018). Perceived fatigability/perception of fatigue can refer to a feeling of reduced capacity to cope with physical or mental stressors (Micklewright et al., 2017) and is related to the maintenance of homeostasis and the psychological state of the individual (Kluger et al., 2013; Enoka and Duchateau, 2016). Performance fatigability refers to a decline in an objective measure of performance (e.g., muscle force or power) during and/or after a given exercise, hereafter referred to as fatigability. Although linked but not interchangeable, fatigability should not be confused with another commonly-used term when exercise-induced fatigue is investigated, i.e., time to task failure (TTF). This term refers to the capacity for a subject to perform an exercise at a given percentage of a maximal parameter (e.g., muscle force, maximal aerobic power) over an extended period of time until failure.

Over the last three decades, several studies investigated differences in TTF [e.g., Berthoin et al. (2003); Barker et al. (2010); Leclair et al. (2011); Patikas et al. (2013); Hatzikotoulas et al. (2014); Tanina et al. (2017); Piponnier et al. (2018); Bar-Yoseph et al. (2019)] and fatigability [Pullinen et al. (2011); Patikas et al. (2013); Hatzikotoulas et al. (2014); Murphy et al. (2014); Willcocks et al. (2014); Lazaridis et al. (2018)] between youth (that includes both children and adolescents) and adults. Of note, the current literature has rarely considered the adolescents versus children and/or adults comparison for the evaluation of fatigability. While it seems that children and adolescents have lower level of fatigability and longer TTF than adults, the lack of consistency in the experimental procedures prevent an appropriate interpretation. For instance, while some studies used experimental designs where the exercise duration (or the number of contractions in the case of intermittent exercises) was fixed, e.g., a 30-s sustained maximal voluntary contraction (MVC) (Halin et al., 2003; Streckis et al., 2007; Hatzikotoulas et al., 2009; Gorianovas et al., 2013; Lazaridis et al., 2018), many other studies used protocols with a pre-set amount of fatigability, e.g., the exercise stopped when the subject reached a decrease of 40% of the baseline force level (Armatas et al., 2010; Hatzikotoulas et al., 2014; Piponnier et al., 2018; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020), thus preventing any comparison of fatigability between youth and adults. To allow a reliable comparison for the level of fatigability between youth and adults, studies should report an "isotime" measurement that includes only the portion of the fatiguing exercise that is available for all the subjects being analyzed, which is limited by the subject with the shortest TTF (Nicolò et al., 2019). This specific method of analysis allows to compare youth versus adults at a similar exercise duration, without any consideration of the total TTF that could largely differ between these two populations (Armatas et al., 2010; Barker et al., 2010; Hatzikotoulas et al., 2014; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b).

While various fatiguing protocols have been used to assess either TTF and/or fatigability in youth and adults, one should note that the exercise modality may influence the reported differences in these two concepts when these two populations are compared. First, the type of exercise, i.e., dynamic (e.g., cycling, running, jumping, isokinetic contractions) versus isometric exercises, should be considered when looking at potential between-group differences in fatigability and/or TTF. This issue has not been directly addressed so far and a critical review of the literature could help to better understand how the type of exercise could influence the potential differences in fatigability and TTF between youth and adults. This is an important question since large differences in physiological demands exist between these two types of exercise. For instance, performing either a dynamic or isometric exercise may modulate the influence of blood flow occlusion on exercise performance. Intramuscular pressure is dramatically increased during isometric compared to dynamic exercises (Lind, 2011). This could lead to large variations in the stimulation of type III/IV metabo-nociceptive afferents, which project their inputs to various sites within the central nervous system then modulating exercise performance (Amann et al., 2011; Hureau et al., 2018). The consideration of two populations with fundamental differences in physiological functioning (Ratel and Blazevich, 2017; Patikas et al., 2018) may exacerbate the effect that blood flow occlusion could have on the level of fatigability and TTF depending on the type of exercise that is used. For instance, large differences in muscle mass may have a direct impact on intramuscular pressure and blood flow occlusion during exercise. Second, one may question the influence that exercise duration could have in the differences in TTF and fatigability between youth and adults, i.e., do differences in TTF and/or fatigability between children and adults are greater or lower for short or long-duration exercises? Indeed, exercise duration could influence the mechanisms responsible for impairments in exercise performance (Brownstein et al., 2020). Longer exercise duration usually leads to a larger magnitude of fatigability (e.g., greater loss of maximal force), as reported for instance in running exercises (Temesi et al., 2021). Because the contribution of each energy systems (i.e., aerobic versus

anaerobic metabolisms) in exercise performance may differ between youth and adults, it is tempting to suggest that differences in fatigability and TTF between youth and adults may be influenced by exercise duration. Such a question has never been studied so far, and a quantitative analysis of the current literature could help to shed light on this specific point.

The primary purpose of this study is to systematically review the literature regarding the differences in fatigability and TTF between youth (including children and adolescents) and young adults (18–35 years old) and to assess the influence of exercise modalities on these outcomes. The secondary aim of this review is to identify the physiological mechanisms underlying the reported differences between youth and adults, based on neuromuscular evaluation (i.e., central and peripheral components of fatigue).

2 Materials and methods

2.1 Literature search strategy

We looked for cross-sectional and longitudinal studies that compared TTF and fatigability between youth (i.e., including both children and adolescents) and young adults (18–35 years old). A distinction is made throughout the manuscript between children and adolescents, with the former and the latter referring to prepubescent and pubescent young individuals, respectively. We made this distinction based on objective criteria displayed in the articles, e.g., Tanner classification and/or peak height velocity. When no objective criteria were given, we arbitrary classified the data in the children category (knowing that it could include both prepubescent and pubescent young individuals). We used the following electronic databases: Medline (*via* PubMed), SPORTDiscus and Cochrane Library. Each database was searched from inception until 5 June 2020. The search was conducted by combining terms related to the intervention (e.g., whole-body or isometric exercises), population (e.g., child, adolescent, prepubertal, pubertal) and outcomes (e.g., fatigability, isometric and/or dynamic force, power, endurance, number of contractions, time to exhaustion). There was a language restriction (English or French) and only accepted or published studies were considered. Details of the protocol and search strategy (Supplementary Table S1, Supplemental Digital Content 1, which displayed detailed information about our search strategy) for this systematic review were registered on PROSPERO (CRD42020184549).

2.2 Selection of studies

The initial search was performed by two authors (RS and MG). The first step consisted in screening titles and abstracts. The articles that were judged to be outside the scope of this meta-analysis were removed. Following this first screening, and in the case the abstracts did not provide enough information, two authors (RS and MG) independently selected and reviewed all included articles. At this point, all duplicate studies were removed. The articles that met the inclusion criteria were read and eligible studies were included in the meta-analyses (Figure 1). Disagreements were solved by a third author (PD).

2.3 Eligibility criteria—inclusion and exclusion

Studies were considered for review if they met the following PICOS (i.e., Population, Intervention; Comparison group; Outcomes; Study type) criteria: 1) Comparison between youth (<18 years) and adults (18–35 years); 2) existence of a fatiguing exercise protocol (i.e., dynamic or isometric); 3) assessment of fatigability (i.e., evaluated by changes in muscle force and/or power and/or velocity after the fatiguing exercise) and/or TTF (i.e., evaluated by a TTF in the case of continuous exercises or a number of repetitions in the case of intermittent exercises); 4) cross-sectional and longitudinal studies (in the case of longitudinal studies that assessed the effect of a training intervention, only baseline data were included). The exclusion criteria were 1) a lack of a comparison group (i.e., adult group); 2) an absence of investigation of the main outcomes of interest for the meta-analysis (i.e., isometric or dynamic muscle force, power output, maximal velocity, TTF, number of contractions); 3) an adult group older than 35 years; 4) any publications written in another language than English or French.

2.4 Quality assessment

The methodological quality assessment of all studies included in the meta-analysis was performed with a modified Newcastle-Ottawa Quality Assessment Scale for cross-sectional studies. This scale is based on three broad criteria that are specific to the study design, i.e., 1) selection of study groups; 2) comparability and 3) outcome assessments. Similarly to a recent meta-analysis that investigated the differences in fatigability between healthy young and old subjects (Kruger et al., 2018), we modified the original quality scale to meet the needs of our study design (Supplementary Material S2, Supplemental Digital Content 2 which displayed the items for the quality assessment of the included studies). First, the selection domain presents three sub-categories, i.e., the representativeness of the sample (is the sample representative of the average in the target population or was it a sample from selected group of users?), the sample size (is there a justification for sample size calculation?) and the ascertainment of participants' health status (is the participants' health status checked with medical report or specific questionnaire or is the participants' health status only basically reported?). As

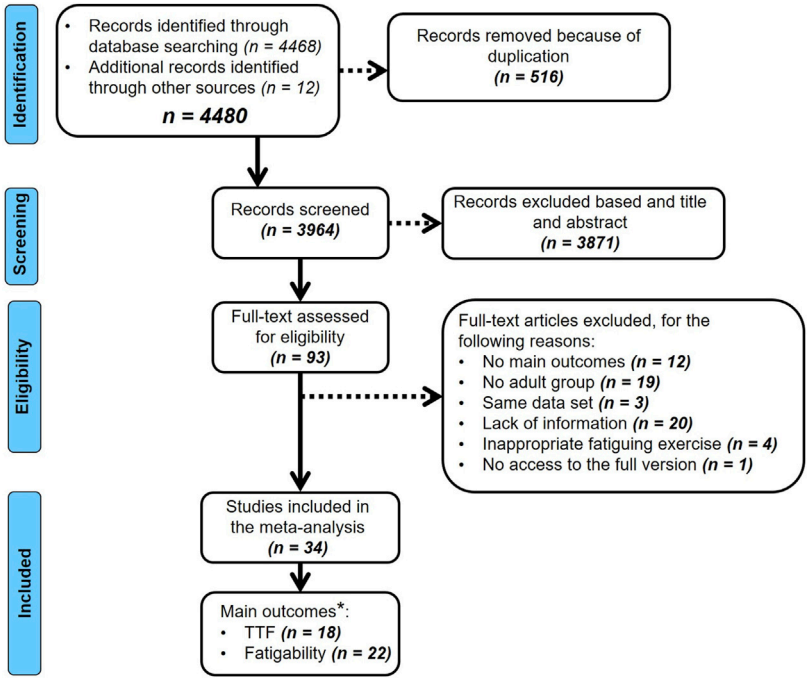

FIGURE 1
PRISMA flowchart of included studies. *Some articles investigated TTF and fatigability in the same experimental design and were included in both meta-analyses.*

performed by Kruger et al. (2018), the ascertainment of exposure section from the original scale has been adapted to our study design to have information on participants' health status. Second, the comparability domain allowed us to control for physical activity and fitness levels and to control for any additional factors that could have impacted the main outcomes (e.g., caffeine and alcohol consumption, strenuous physical activity before physical testing). Third, the outcome domain presents two sub-categories, i.e., the assessment of the main outcomes (are the main outcomes obtained after reliable fatiguing protocol and using validated measuring tools?) and the statistical test (is the statistical test used to analyze the data clearly described and appropriate?). The quality of the paper was rated by stars, ranking from zero to four for the selection domain, zero to two for the comparability domain and zero to three for the outcome domain, for a maximum of nine stars (one being least quality and nine maximum quality). The global quality scores were calculated based on the scoring algorithm proposed by McPheeters et al. (2012).

2.5 Data extraction

For all studies included in the meta-analysis, study characteristics (i.e., authors, year, sample size, study design), participant' demographics (i.e., age, sex), fatiguing exercise details (i.e., isometric or dynamic fatiguing task, muscle(s) involved in the exercise) and main outcomes (i.e., muscle force and/or power and/or velocity, TTF, number of contractions) were retrieved on a standardized Excel sheet. Corresponding authors were contacted when data were missing. Different meta-analyses were performed for the main outcomes (see the sections below).

2.5.1 Time to task failure

The first meta-analyses compared TTF between children versus adults and adolescents versus adults. In the case of sustained prolonged exercises, the total time (in seconds) was extracted for further analysis. In the case of intermittent exercises, and if the article only reported the total number of contractions until task failure, the data were extracted and transformed in time units (i.e., seconds). The resting time allowed between each intermittent contraction (e.g., 5 s ON/5 s OFF) was included in the calculation to obtain the total exercise duration. If these information (i.e., number of contractions, duration of the contraction, resting time allowed between contractions) were missing, the article was not included in these meta-analyses. Further, studies were excluded for TTF-related meta-analysis (but not fatigability analysis, see below) if the exercise was performed with a fixed duration (e.g., a sustained

submaximal isometric contraction for 10 min) or a fixed number of contractions (e.g., 50 intermittent contractions with 5 s ON/5 s OFF). When more than one fatiguing exercise was performed in a similar study (e.g., sustained contraction at 20 and 60% MVC), the data obtained during the longest exercise was kept for quantitative analysis.

2.5.2 Performance fatigability

Additional meta-analyses compared the indices of fatigability (i.e., muscle force, power and/or velocity) between children versus adults and adolescents versus adults. The analyses were only performed for data extracted from studies that used an experimental design where the exercise duration or the number of contractions were fixed (e.g., a sustained maximal isometric contraction maintained for 2 min or a similar number of intermittent contractions performed by participants over a similar period of time). When a study did not meet these criteria (e.g., a study where exercise termination was set to a 30% decrease in maximal force, independently of the time to reach this target), the authors were contacted to know if they performed an "isotime" comparison, i.e., the analysis that includes only the portion of the exercise that is available for all the participants in the groups being analyzed, which is limited by the participant with the shortest TTF (Nicolò et al., 2019), or if they were willing to perform such analyses. In such case, these data were included in the meta-analyses. Otherwise, the article was excluded from the meta-analyses. Because huge differences exist in baseline maximal muscle force and power between children and adults, these meta-analyses solely included the studies that reported relative data (i.e., when the changes at the end of the fatiguing exercise were expressed as a percentage of the baseline value). When only absolute data (i.e., Newton for muscle force and Watts for muscle power) were reported, we contacted the authors and asked them to provide the relative data.

2.5.3 Exercise modalities

Separate subgroup meta-analyses were performed to investigate the potential influence of exercise modality on the reported differences in TTF and fatigability between children and adults. For that purpose, we dissociated the type of exercises that were performed, i.e., isometric versus dynamic (e.g., running, cycling, jumping, isokinetic contraction). We also investigated the potential influence of exercise duration on child-adult differences in TTF and fatigability. For this purpose, the median was calculated and the studies were classified either as long (i.e., duration > median) or short (i.e., duration < median) duration.

2.5.4 Peripheral and central components of fatigue

When available, data on central and peripheral factors of fatigue were also extracted as secondary outcomes to shed light on the potential differences in TTF and fatigability between children and adults. The following parameters were considered to investigate central factors of fatigue: 1) Voluntary activation level (VA), consisting in an electrical/magnetic stimulation superimposed to a maximal voluntary contraction (both the interpolated twitch technique and the central activation ratio were considered), 2) normalized electromyographic (EMG) signals recorded during a maximal force; 3) transcranial magnetic stimulation (TMS)-related parameters, i.e., motor evoked potentials (MEP, amplitude and/or area) and silent period duration to investigate corticospinal excitability and inhibition, respectively; 4) H- and T-reflexes to investigate spinal excitability. The following parameters were considered to investigate peripheral factors of fatigue: 1) peak twitch (Pt) and doublet (Db), i.e., the mechanical response to a single or double electrical and/or magnetic stimulation, with its associated characteristics (e.g., half relaxation time, time to Pt) also being considered; 2) M-wave amplitude and/or area, i.e., the EMG response to a single electrical and/or magnetic stimulation; 3) low-to-high frequency fatigue ratio (LHF_R), i.e., the ratio of peak forces evoked by low and high-frequency doublets or stimulation trains (e.g., the ratio between Db evoked at 10 and 100 Hz).

2.6 Data analysis

Descriptive statistics (mean, median, range) were used to describe studies characteristics and methodological quality of all the included studies.

Hedges' g were calculated (Hedges and Olkin, 1985) as the measure of standardized mean difference (SMD), i.e., the difference between the outcome mean values of the children or adolescents and the adult group divided by the pooled standard deviation (Hedges and Olkin, 1985; Morris and DeShon, 2002). A negative SMD indicates less fatigability or TTF in children, whereas a positive SMD represents greater fatigability or TTF in adults. To assess the difference in outcomes of interest between children and adults, effect sizes were estimated by weighting SMDs by the inverse of their variance based on random effects models (Shadish and Haddock, 2009).

Heterogeneity was tested with Cochran's chi-square test (Q) to assess the consistency of associations. To quantify the extent of heterogeneity, we estimated the between-study variance (I^2). I^2 statistic describes the proportion of variability in SMDs due to the heterogeneity between studies ranging from 0% to 100% (with small heterogeneity: < 25%; moderate: 25–50%; high: ≥ 50%). Because heterogeneity was high (I^2 > 50%), random effect models were used to incorporate heterogeneity in meta-analyses (Higgins et al., 2003).

In addition, effect sizes were computed for subgroups of included studies based on dichotomized identified exercise modalities that may impact our main outcomes, i.e., type (isometric versus dynamic) and duration of exercise (<

TABLE 1 Study characteristics—Fatigability.

Study	Participant's age (*n*)			Fatiguing exercise (*criteria for exercise ending*)	Main outcome (investigated muscle)	% decrease			
	Ch	Ado	Adu			Ch	Ado	Adu	Statistical significance
ISOMETRIC EXERCISE									
Bontemps et al. (2019)	10 (18)	—	22 (19)	5 s MVC/5 s rest (↓ *of 40% MVC*)	I_{MVC} (KE)	**38**	—	**49**	S
Halin et al. (2003)	11 (15)	—	22 (12)	30-s sustained MVC	I_{MVC} (EF)	**29**	—	**35**	S
Hatzikotoulas et al. (2009)	10 (15)	—	24 (15)	10-min sustained 20% MVC	I_{MVC} (PF)	**24**	—	**26**	NS
Piponnier et al. (2020)	10 (19)	—	22 (23)	5 s MVC/5 s rest optimal length (↓ *of 40% MVC*)	IMVC (PF)	**36**	—	**22**	S
				5 s MVC/5 s rest long length (↓ *of 40% MVC*)		**34**	—	**25**	S
				5 s MVC/5 s rest short length (↓ *of 40% MVC*)		**35**	—	**26**	S
Piponnier et al. (2019b)	10 (22)	—	21 (22)	5 s MVC/5 s rest optimal length (↓ *of 40% MVC*)	I_{MVC} (KE)	**25**	—	**34**	S
				5 s MVC/5 s rest long length (↓ *of 40% MVC*)		**27**	—	**23**	NS
				5 s MVC/5 s rest short length (↓ *of 40% MVC*)		**24**	—	**22**	NS
Piponnier et al. (2019a)	10 (9)	14 (8)	24 (11)	5 s MVC/5 s rest (↓ *of 40% MVC*)	I_{MVC} (KE)	**20**	**25**	**32**	S
Ratel et al. (2015)	10 (11)	—	24 (12)	5 s MVC/5 s rest (↓ *of 40% MVC*)	I_{MVC} (KE)	**22**	—	**30**	S
Willcocks et al. (2014)	—	13 (6)	29 (6)	30 × 6 s MVC	I_{MVC} (KE)	—	**23**	**31**	NS
DYNAMIC EXERCISE									
Äyrämö et al. (2017)	12 (8)	15 (8)	21 (8)	50-s all out run	I_{MVC} (PF)	**3**	**+7**	**16**	NS (Ch vs. Adu) S (Ado vs. Adu)
Birat et al. (2018)	10 (12)	—	21 (12)	30-s Wingate test	MP (LL)	**35**	—	**52**	S
Buchheit et al. (2010)	10 (10)	15 (6)	20 (7)	10 × 10-s all-out cycling sprint	MP (LL)	**6**	**6**	**5**	NS
De Ste Croix et al. (2009)	12 (16)	–	30 (24)	50 CONC MVC	D_{MVC} (KE)	**39**	—	**60**	S
Dipla et al. (2009)	11 (10)	15 (10)	24 (10)	4 × 18 KE-KF CONC MVC	D_{MVC} (KE)	**9**	**16**	**29**	S (Ch & Ado vs. Adu)
					D_{MVC} (KF)	**NC**	**15**	**23**	S (Ch vs. Ado & Adu)
Ftikas et al. (2010)	10 (11)	—	24 (11)	10 × 10 max vertical jumps	I_{MVC} (KE)	**13**	—	**18**	S
Gorianovas et al. (2013)	12 (11)	—	21 (11)	100 max drop jumps	I_{MVC} (KE)	**22**	—	**36**	NR
Hebestreit et al. (1993)	10 (8)	—	22 (8)	30-s Wingate test	MP (LL)	**44**	—	**52**	S
Kanehisa et al. (1995)	—	14 (26)	18–25 (27)	50 CON MVC	D_{MVC} (KE)	—	**36**	**48**	S
Lazaridis et al. (2018)	10 (13)	—	25 (13)	10 × 10 max CMJ	I_{MVC} (KE)	**12**	—	**18**	S
Liamopoulou et al. (2015)	10 (12)	—	25 (12)	10 × 10 max plyometric jumps	I_{MVC} (KE)	**14**	—	**22**	S

(Continued on following page)

TABLE 1 (*Continued*) Study characteristics—Fatigability.

Study	Participant's age (*n*)			Fatiguing exercise (*criteria for exercise ending*)	Main outcome (investigated muscle)	% decrease			
	Ch	Ado	Adu			Ch	Ado	Adu	Statistical significance
Marginson et al. (2005)	10 (10)	—	22 (10)	8 × 10 max plyometric jumps	I_{MVC} (KE)	**14**	—	**26**	S
Pullinen et al. (2011)	—	14 (6)	27 (6)	5 × 10 contractions 40% RM	I_{MVC} (KE)	—	**15**	**25**	NS
Weinstein et al. (2018)	10 (11)	—	20 (10)	30-s Wingate test	MP (LL)	**33**	—	**47**	S

Data presented in bold black were given as relative results (PRE-POST, changes in % of PRE) in the published article. Data presented in bold blue were calculated by the authors from the absolute results given in the article. In the cases where data were not fully presented in the manuscript, data were extracted from original figures using ImageJ software (ImageJ V.1.45 s, National Institute of Health, MD, United States). Ado, adolescents; Adu, adult; BP, bench press; Ch, children; CON, concentric; D_{MVC}, dynamic maximal voluntary contraction; ECC, eccentric; EF, elbow flexors; I_{MVC}, isometric maximal voluntary contraction; KE, knee extensors; KF, knee flexors; LL, lower limbs; MP, muscle power; MVC, maximal voluntary contraction; n, number of participants; NC, no change; NR, not reported; NS, nonsignificant (i.e., $p > 0.05$); PF, plantar flexors; RM, maximum repetition; S, statistically significant (i.e., $p < 0.05$).

median versus ≥ median duration across studies). Publication bias was searched by funnel plot representation and Egger's (Egger et al., 1997) and Begg's (Begg and Mazumdar, 1994) tests with $p < 0.10$ taken as an indication of publication bias. All statistical analyses were carried out by using Stata software version 11 (StataCorp, College Station, TX, United States).

3 Results

The process of study identification, screening, and evaluation of the eligibility of included studies is displayed by the PRISMA flow chart (Figure 1). The initial searches provided a total of 4,468 articles. Following the removal of duplicates, the titles and abstracts of the remaining 3,964 records were screened, with 3,871 being excluded at this stage for not meeting the inclusion criteria. Then, full texts of 93 records were assessed for eligibility with a further 59 of these being removed for various reasons (i.e., lack of main outcomes, lack of adult group, same data set, inappropriate fatiguing exercise, no access to the full version of the article, lack of information that includes for instance studies reported only absolute rather than relative data for the fatigability domain). This leaves 34 records that were included in the meta-analyses. A detailed description of the characteristics of the meta-analyzed studies that investigated differences in TTF and fatigability between children, adolescents and adults is given in Tables 1, 2, respectively.

3.1 Quality assessment

Studies that met the inclusion criteria ranged between two and seven stars (one being least quality and nine maximum quality), with a mean score of 4.3 ± 1.2 and a median of 4 (Supplementary Table S3, Supplemental Digital Content 3 which presents the detailed results for quality assessment of the included studies). Considering the classification proposed by McPheeters et al. (2012) and regarding the risk of bias, 0% (0/29), 28% (8/29) and 72% (21/29) of the included studies had a good, fair and poor grade, respectively. For the comparability domain, 21% (6/29), 55% (16/29) and 24% (7/29) of the studies had a good, fair and poor grade, respectively. Finally, for the outcome domain, 17% (5/29), 76% (22/29) and 7% (2/29) of the studies had a good, fair and poor grade, respectively.

3.2 Publication bias

Regarding TTF, no evidence of publication bias was identified by Begg's and Egger's tests ($p > 0.10$) or funnel plot representation (Supplementary Figure S1, Supplemental Digital Content 4). However, Begg's and Egger's tests indicated evidence for small study-effects in fatigability with $p = 0.030$ and 0.025, respectively. Funnel plot representation (Supplementary Figure S2, Supplemental Digital Content 5) showed a little asymmetry with a few studies with relatively small sample size reporting the largest effects on fatigability in favor of children as compared to adults. A risk of publication bias for fatigability suggests that our analyses may be biased in the sense of an overestimation of the fatigability differences between children and adults.

3.3 Time to task failure

Two separate meta-analyses compared TTF between children versus adults and adolescents versus adults. The exercise

TABLE 2 Study characteristics—Time to task failure.

Study	Participant's age (*n*)			Fatiguing exercise (*criteria for exercise ending*)		TTF (in s)			
	Ch	Ado	Adu		Muscle group	Ch	Ado	Adu	Statistical significance
ISOMETRIC EXERCISE									
Armatas et al. (2010)	10 (13)	—	26 (13)	5 s MVC/5 s rest (↓ *of 50% MVC*)	KE	**563**	—	**348**	S
Bontemps et al. (2019)	10 (18)	—	22 (19)	5 s MVC/5 s rest (↓ *of 40% MVC*)	KE	**404**	—	**159**	S
Hatzikotoulas et al. (2014)	11 (10)	—	26 (11)	Sustained MVC (↓ *of 50% MVC*)	PF	**127**	—	**94**	S
Patikas et al. (2013)	10 (14)	—	24 (14)	Sustained 20% MVC (5 *s < 95% target force*)	PF	**771**	—	**786**	NS
				Sustained 60% MVC (5 *s < 95% target force*)		**195**	—	**201**	NS
Piponnier et al. (2020)	10 (19)	—	22 (23)	5 s MVC/5 s rest optimal length (↓ *of 40% MVC*)	PF	**156**	—	**135**	NS
				5 s MVC/5 s rest long length (↓ *of 40% MVC*)		**120**	—	**130**	NS
				5 s MVC/5 s rest short length (↓ *of 40% MVC*)		**170**	—	**160**	NS
Piponnier et al. (2019b)	10 (22)	—	21 (22)	5 s MVC/5 s rest optimal length (↓ *of 40% MVC*)	KE	**397**	—	**148**	S
				5 s MVC/5 s rest long length (↓ *of 40% MVC*)		**295**	—	**158**	S
				5 s MVC/5 s rest short length (↓ *of 40% MVC*)		**337**	—	**409**	NS
Piponnier et al. (2019a)	10 (9)	14 (8)	24 (11)	5 s MVC/5 s rest (↓ *of 40% MVC*)	KE	**529**	**426**	**266**	S (Ch & Ado vs. Adu)
Ratel et al. (2015)	10 (11)	—	24 (12)	5 s MVC/5 s rest (↓ *of 40% MVC*)	KE	**495**	—	**340**	S
Tanina et al. (2017)	9 (14)	—	25 (14)	Sorensen back test (> *2 cm reduction in height for 2 s*)	TE	**95**	—	**98**	NS
Woods et al. (2019)	10 (18)	—	24 (21)	Intermittent 5 s submaximal contractions (*volitional exhaustion*)	KE	**688**	—	**632**	NS
Woods et al. (2020)	10 (17)	—	24 (17)	Intermittent 5 s submaximal contractions (*volitional exhaustion*)	KE	**608**	—	**560**	NS
DYNAMIC EXERCISE									
Bar Yoseph et al. (2019)	11 (18)	17 (18)	29 (8)	Incremental cycling exercise (*cadence < 60 rpm*)	LL	**670**	**692**	**690**	NS
				Incremental running exercise (*volitional exhaustion*)		**700**	**951**	**1,013**	S (Ado & Adu vs. Ch)
Berthoin et al. (2003)	11 (9)	—	22 (8)	Cycling at 120% PMA (*volitional exhaustion*)	LL	**53**	—	**122**	S
Leclair et al. (2011)	10 (15)	—	24 (15)	Constant load cycling exercise P50 (*cadence < 70 rpm*)	LL	**702**	—	**754**	NS
				Constant load cycling exercise P75 (*cadence < 70 rpm*)		**307**	—	**371**	
				Constant load cycling exercise P100 (*cadence < 70 rpm*)		**144**	—	**221**	S
				Constant load cycling exercise P110 (*cadence < 70 rpm*)		**96**	—	**147**	S
Murphy et al. (2014)	10 (10)	—	26 (10)	3 × max CON MVC Low RM (*volitional exhaustion*)	KE	**274**	—	**253**	S
				3 × max CON MVC High RM (*volitional exhaustion*)		**213**	—	**193**	
Pullinen et al. (2011)	—	14 (8)	31 (8)	3 × max contractions at 40% RM (*volitional exhaustion*)	KE	—	**48**	**48**	NS
Pullinen et al. (2002)	—	14 (6)	27 (6)	1 × max contractions at 40% RM (*volitional exhaustion*)	KE	—	**46**	**42**	NS
Tibana et al. (2012)	—	15 (15)	22 (15)	3 × max chest press w/30 s rest (*volitional exhaustion*)	UL	—	**96**	**88**	S
				3 × max chest press w/60 s rest (*volitional exhaustion*)		—	**163**	**156**	S
				3 × max chest press w/120 s rest (*volitional exhaustion*)		—	**292**	**282**	S

Data presented in bold black were directly given in time units (seconds) in the article. Data presented in bold blue were calculated by the authors from the number of contractions performed during the fatiguing exercise. Ado, adolescents; Adu, adult; Ch, children; CON, concentric; KE, knee extensors; KF, knee flexors; LL, lower limbs; MVC, maximal voluntary contraction; n, number of participants; NS, nonsignificant; P50 and P75, intensities corresponded to 50 and 75% of the difference between maximal aerobic power and the power associated with the ventilatory threshold; P100 and P110, intensities corresponded to 100 and 110% of maximal aerobic power; PF, plantar flexors; RM, maximum repetition; S, statistically significant (i.e., $p < 0.05$); TE, trunk extensors; TTF, time to task failure; UL, upper limbs.

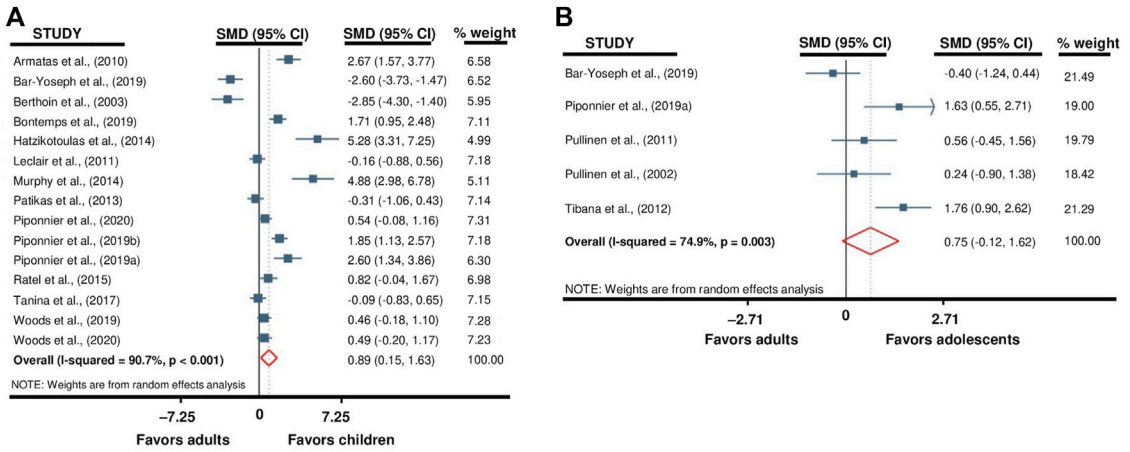

FIGURE 2
Forest plot from the meta-analysis reporting TTF differences between children versus adults **(A)** and adolescents versus adults **(B)**.

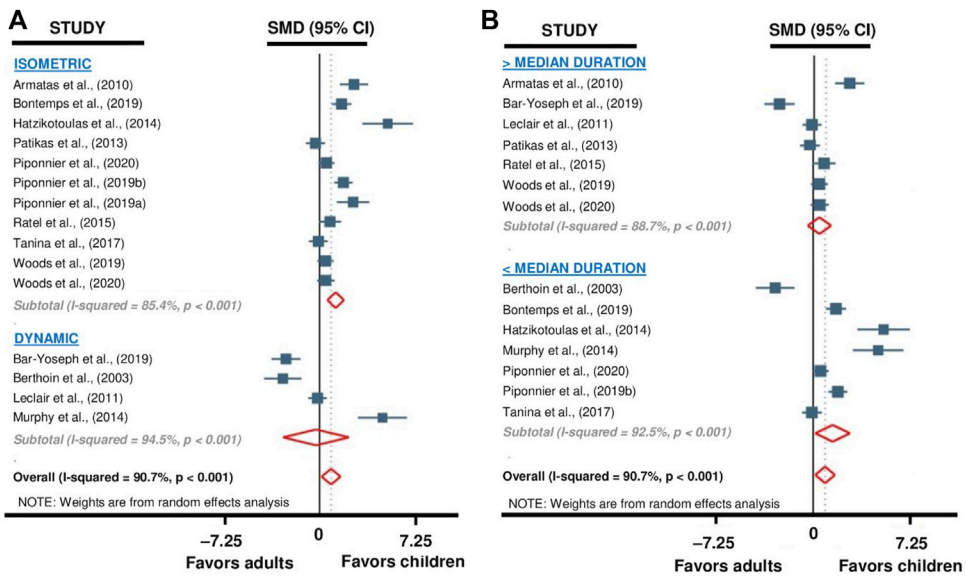

FIGURE 3
Forest plot from the subgroup meta-analysis reporting the influence of 1) exercise modality (i.e., isometric or dynamic; **(A)** and 2) exercise duration **(B)** on TTF differences between children and adults. Raw SMDs (with 95% confidence intervals) and % weights of the meta-analyzed studies are given in Figure 2.

duration of 12 out of 18 (67%) of the studies included in these meta-analyses (Pullinen et al., 2002; Berthoin et al., 2003; Armatas et al., 2010; Pullinen et al., 2011; Tibana et al., 2012; Murphy et al., 2014; Ratel et al., 2015; Piponnier et al., 2019a; Piponnier et al., 2019b; Woods et al., 2019; Piponnier et al., 2020; Woods et al., 2020) was derived from the total number of contractions performed until task failure. The TTF in seconds was directly provided in the other six studies (Berthoin et al., 2003; Leclair et al., 2011; Patikas et al., 2013; Hatzikotoulas et al., 2014; Tanina et al., 2017; Bar-Yoseph et al., 2019). The meta-analyses indicated that TTF was longer in children when compared to adults (SMD 0.89; 95% CI 0.15 to 1.63; p = 0.018; *15 studies*, n = *435*; Figure 2A), with no differences for the adolescents *versus* adults comparison (SMD 0.75; 95% CI −0.12 to 1.62; p = 0.090; *5 studies*, n = *103*; Figure 2B). Heterogeneity of the results was high for children (Q = 150.5;

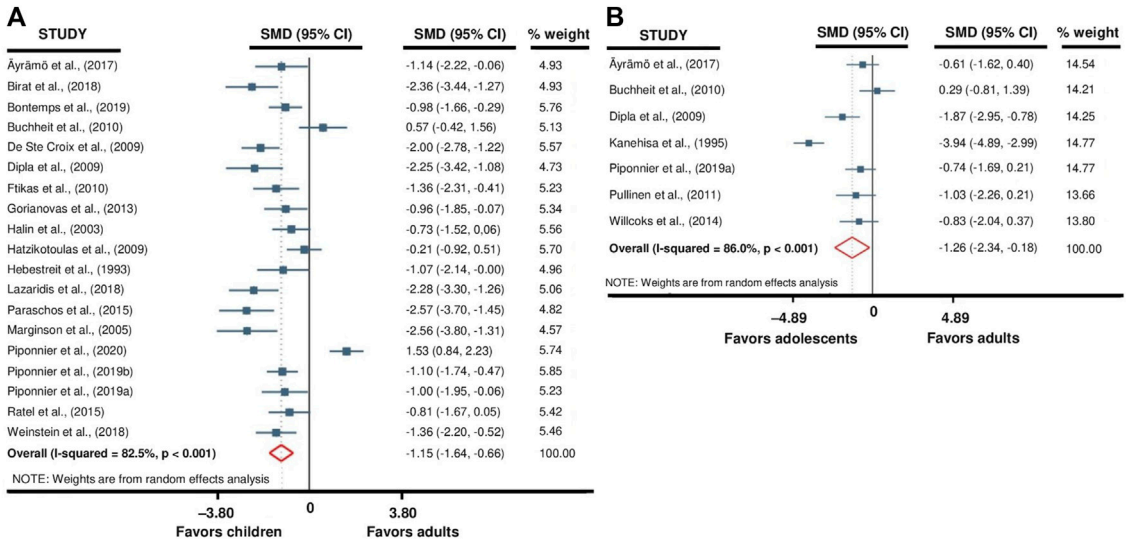

FIGURE 4
Forest plot from the meta-analysis reporting differences in fatigability between children versus adults **(A)** and adolescents versus adults **(B)**.

df = 14; $p < 0.001$; $I^2 = 90.7\%$) and adolescents (Q = 15.9; df = 4; $p = 0.003$; $I^2 = 74.9\%$) versus adults comparison.

A first subgroup meta-analysis was performed to evaluate the influence of the type of exercise (i.e., isometric versus dynamic) on the reported differences in TTF between children and adults. This analysis revealed that children had longer TTF than adults for isometric exercises (SMD 1.25; 95% CI 0.60 to 1.90; $p < 0.001$; *11 studies*, $n = 342$; Figure 3A), but no difference was found for dynamic exercises (SMD −0.27; 95% CI −2.82 to 2.28; $p = 0.83$; *4 studies*, $n = 93$; Figure 3A). The heterogeneity of the results obtained for subgroup isometric (Q = 68.6; df = 10; $p < 0.001$; $I^2 = 85.4\%$) and dynamic (Q = 54.5; df = 3; $p < 0.001$; $I^2 = 94.5\%$) exercises analysis was high. Because of an unbalanced repartition of studies (i.e., one with isometric and four with dynamic modality of exercise), this subgroup analysis was not performed for the adolescents versus adults comparison.

A second subgroup meta-analysis was performed to evaluate the influence of exercise duration on the reported differences in TTF between children and adults. For this analysis, the study with the duration equal to the median (Piponnier et al., 2019a) was excluded, thus 14 studies were included. The mean duration (pooled data of children and adults participants) of the studies in the short-duration and the long-duration categories were 180 ± 89 s (range: 88–282 s) and 640 ± 164 s (range: 418–857 s), respectively. This analysis indicated that differences in TTF were significant between children and adults for short-duration exercises (SMD 1.46; 95% CI 0.16 to 2.76; $p = 0.028$; *7 studies, n = 209*; Figure 3B) while it was not for long-duration ones (SMD 0.20; 95% CI −0.66 to 1.07; $p = 0.64$; *7 studies, n = 206*; Figure 3B). The heterogeneity of the results obtained for the subgroup analysis relative to short- (Q = 80.4; df = 6; $p < 0.001$; $I^2 = 92.5\%$) and long-duration (Q = 48.9; df = 6; $p < 0.001$; $I^2 = 87.7\%$) exercises was high. Because of a too low number of studies (i.e., two versus two studies if the median was excluded), this subgroup analysis was not performed for the adolescents versus adults comparison.

3.4 Fatigability

The meta-analyses that aimed to evaluate the differences in fatigability between children versus adults and adolescents versus adults included 19 and 7 studies, respectively. The analyses revealed that children (SMD −1.15; 95% CI −1.64 to −0.66; $p < 0.001$; *19 studies*, $n = 489$; Figure 4A) and adolescents (SMD −1.26; 95% CI −2.34 to −0.18; $p = 0.022$; *7 studies*, $n = 149$; Figure 4B) were significantly less fatigable when compared to adults, with the heterogeneity of studies being high either for the children versus adults (Q = 103.1; df = 18; $p < 0.001$; $I^2 = 82.5\%$) or adolescents versus adults (Q = 42.8; df = 6; $p < 0.001$; $I^2 = 86.0\%$) comparisons.

A subgroup analysis was performed to evaluate the influence of the type of exercise on the reported differences in fatigability between children and adults. This subgroup analysis was not performed on adolescents because of a too low number of studies. The subgroup meta-analysis reported that children were significantly less fatigable than adults during dynamic exercises (SMD −1.58; 95% CI −2.08 to −1.08; $p < 0.001$; *12 studies, n = 275*; Figure 5), but non-significant difference was found for isometric exercises (SMD −0.46; 95% CI −1.19 to 0.27; $p = 0.22$; *7 studies*, $n = 214$; Figure 5). The heterogeneity of the studies involved in isometric (Q = 41.8; df = 7; $p < 0.001$; $I^2 = 83.2\%$) and dynamic (Q = 32.7; df = 10; $p < 0.001$; $I^2 = 69.5\%$)

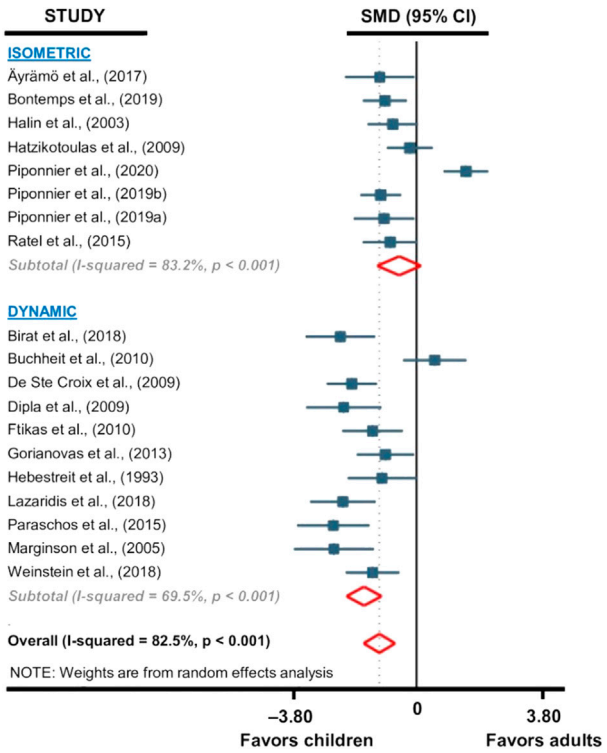

FIGURE 5
Forest plot from the subgroup meta-analysis reporting the influence of exercise modality (i.e., isometric or dynamic) on fatigability differences between children and adults. Raw SMDs (with 95% confidence intervals) and % weights of the meta-analyzed studies are given in Figure 4.

exercises subgroups analyses was high. Because of too low number of studies (i.e., three *versus* four studies), this subgroup analysis was not performed for the adolescents versus adults comparisons.

3.5 Secondary outcomes

Out of the 34 studies included in the meta-analyses, eight (Hatzikotoulas et al., 2014; Murphy et al., 2014; Ratel et al., 2015; Äyrämö et al., 2017; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020) used neurostimulation techniques to shed light on the central and peripheral factors that may explain the differences in TTF and fatigability between children and adults. Two additional studies (Streckis et al., 2005; Streckis et al., 2007) that were not included in the previous meta-analyses (i.e., because relative data have not been provided by the authors) also investigated the central and peripheral components of fatigability in both children and adults after exercise. Detailed information on these studies is given in Table 3. Because of the small number of studies and the large heterogeneity in the methods used to investigate the peripheral and central factors underlying fatigability, no meta-analyses were performed.

3.5.1 Central fatigue

Eight out of the ten studies investigated central fatigue, either using VA (Streckis et al., 2007; Hatzikotoulas et al., 2014; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020) or the EMG/M-wave ratio (Äyrämö et al., 2017; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020) or H-reflexes (Äyrämö et al., 2017; Piponnier et al., 2020). Six studies reported that VA was significantly more decreased at the end of the fatiguing exercise in children than in adults (Streckis et al., 2007; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020), while one study reported no significant differences in VA changes with fatigue between children and adults (Hatzikotoulas et al., 2014). Three studies reported that the decrease in the EMG/M-wave ratio was significantly more pronounced in children than adults (Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b). Two studies reported that changes in EMG/M-wave ratio were similar after the exercise between the two populations (Äyrämö et al., 2017; Piponnier et al., 2020), while one study reported no changes in EMG-M-wave ratio after fatigue for both children and adults (Ratel et al., 2015). Finally, two studies reported similar changes in H-reflexes after fatigue in children and adults (Äyrämö et al., 2017; Piponnier et al., 2020).

TABLE 3 Central and peripheral fatigue parameters.

	Fatiguing exercise (*criteria for exercise ending*)	Central factors			Peripheral factors					
		VA/CAR	EMG/ M-wave	H-reflex	Pt	HRT	TPT	M-wave	Db	LHF_R
Äyrämö et al. (2017)	50-s all out run	—	↑ Adu = Ch	↑ Adu = Ch	↓ Adu > Ch	↓ Adu = Ch	↓ Adu = Ch	↓ Adu = Ch	—	—
Bontemps et al. (2019)	5 s MVC/5 s rest (↓ *of 40% MVC*)	↓ Ch > Adu	↓ Ch > Adu	—	↓ Adu > Ch	—	—	NC	↓ Adu > Ch	↓ Adu > Ch
Hatzikotoulas et al. (2014)	Sustained MVC (↓ *of 50% MVC*)	↓ Ch = Adu	—	—	↓ Adu > Ch	—	↓ Adu > Ch	↓ Adu = Ch	—	—
Murphy et al. (2014)	3 × max CON MVC (*volitional exhaustion*)	—	—	—	↓ Adu > Ch	↑ Adu > Ch	—	↓ Adu > Ch	—	—
Piponnier et al. (2020)	5 s MVC/5 s rest (↓ *of 40% MVC*)	↓ Ch > Adu	↓ Ch = Adu	↓ Ch = Adu	↓ Adu > Ch	—	—	↑ Adu > Ch	↓ Adu > Ch	↓ Adu > Ch
Piponnier et al. (2019b)	5 s MVC/5 s rest (↓ *of 40% MVC*)	↓ Ch > Adu	↓ Ch > Adu	—	↓ Adu > Ch	—	—	NC	↓ Adu > Ch	—
Piponnier et al. (2019a)	5 s MVC/5 s rest (↓ *of 40% MVC*)	↓ Ch > Adu	↓ Ch > Adu	—	↓ Adu > Ch ↓ Adu > Ado	—	—	NC	—	—
Ratel et al. (2015)	5 s MVC/5 s rest (↓ *of 40% MVC*)	↓ Ch > Adu	NC	—	↓ Adu > Ch	—	—	NC	—	—
Streckis et al. (2007)	2-min sustained MVC	↓ Ch > Adu	—	—	↓ Adu > Ch	—	—	-	—	—
Streckis et al. (2005)	100 drops jumps	—	—	—	↓ Adu > Ch	—	—	-	—	↓ Adu > Ch

Ado, adolescents; Adu, adult; CAR, central activation ratio; Ch, children; CON, concentric; Db, doublet; EMG, electromyography; HRT, half relaxation time; LHF_R, low-to-high frequency fatigue ratio; MVC, maximal voluntary contraction; Pt, peak twitch; TPT, time to peak twitch; VA, voluntary activation level.

3.5.2 Peripheral fatigue

All studies reported that Pt and Db were significantly more decreased after exercise in adults compared to children (Streckis et al., 2005; Streckis et al., 2007; Hatzikotoulas et al., 2014; Murphy et al., 2014; Ratel et al., 2015; Äyrämö et al., 2017; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020). One study found a greater increase in the half relaxation time (measured from single nerve stimulation) in adults compared to children after exercise (Murphy et al., 2014) while another reported similar changes (Äyrämö et al., 2017). Two studies investigated the time to peak twitch, with one reporting a greater decrease in adults after exercise (Hatzikotoulas et al., 2014) and the other one reporting similar changes between the two populations (Äyrämö et al., 2017). Eight studies investigated M-wave changes with fatigue. One study reported that M-wave decreased more in adults compared to children after exercise (Murphy et al., 2014), while another study reported that M-wave increased in adults after fatigue while it was unchanged in children (Piponnier et al., 2020). The other studies either reported a similar decrease in M-wave between children and adults (Hatzikotoulas et al., 2014; Äyrämö et al., 2017) or no changes in this parameter in both populations (Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020). Lower low-frequency fatigue was found in children compared to adults (Streckis et al., 2005; Bontemps et al., 2019; Piponnier et al., 2020).

4 Discussion

This meta-analysis reveals that children have longer TTF and are less fatigable when compared to adults. Complementary analysis reveals that exercise modality (i.e., exercise duration and type of exercise) influences the differences reported in TTF and fatigability

between children and adults. While this review points out the lack of studies that investigated differences in TTF and fatigability between adolescents *versus* children and adults, the meta-analyses conducted on available data reported a higher fatigability in adults but no differences in TTF between these two populations.

4.1 Children have higher endurance and lower fatigability when compared to adults

Longer TTF were reported in children for ten out of the 15 included studies (Armatas et al., 2010; Hatzikotoulas et al., 2014; Murphy et al., 2014; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Woods et al., 2019; Piponnier et al., 2020; Woods et al., 2020). Seventeen out of the 19 studies that compared fatigability between children and adults observed a lower level of fatigability in children (Hebestreit et al., 1993; Halin et al., 2003; Marginson et al., 2005; De Ste Croix et al., 2009; Dipla et al., 2009; Hatzikotoulas et al., 2009; Ftikas et al., 2010; Gorianovas et al., 2013; Liamopoulou et al., 2015; Ratel et al., 2015; Äyrämö et al., 2017; Birat et al., 2018; Lazaridis et al., 2018; Weinstein et al., 2018; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b). Our results are in line with the current literature and statistically confirm that children have higher endurance capacities than adults (i.e., longer TTF) and are less fatigable (i.e., lower relative decrease in muscle performance at isotime). Physiological differences between children and adults may explain the aforementioned observations (Ratel et al., 2006a).

First, differences in muscle fiber types distribution between children and adults could explain part of the differences in fatigue resistance capacity during exercise. The type of muscle fibers composing a muscle influences its resistance to fatigue, with muscles mainly proportioned in type II fibers being less resistant (Barclay, 1996; Hamada et al., 2003). Although the evidences on age-related muscle typology are scarce so far and obtained on small sample sizes, it has been suggested that children have a higher proportion of slow-twitch type I fibers than adults, as reported by the ~65–70% type I fibers proportion in the vastus lateralis in children versus ~ 47–57% in adults (Lexell et al., 1992; Sjöström et al., 1992). This is associated with a greater muscle oxidative capacity, as demonstrated in the forearm flexor muscles by a higher rate of post-exercise recovery in phosphocreatine and a faster rate of aerobic ATP production (Ratel et al., 2008). Similar results were reported in the gastrocnemius muscle (Taylor et al., 1997). This could have a major influence on fatigue resistance especially during intermittent fatiguing exercise where the recovery in energy substrates plays a key role, i.e., children have a greater ability to replenish their phosphocreatine stores. Also, differences in mitochondrial function and density could favor the children to better liberate and capture oxygen and/or use it (McCormack et al., 2011; Ratel and Blazevich, 2017). Nine out of the 15 meta-analyzed studies that investigated TTF differences between children and adults used experimental designs with intermittent fatiguing protocols (Armatas et al., 2010; Murphy et al., 2014; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Woods et al., 2019; Piponnier et al., 2020; Woods et al., 2020). All of these nine studies reported longer TTF in children, with only three studies reporting the differences to be non-significant. Interestingly, five (Berthoin et al., 2003; Leclair et al., 2011; Patikas et al., 2013; Tanina et al., 2017; Bar-Yoseph et al., 2019) of the six studies that used continuous fatiguing protocols found no differences in exercise duration between children and adults (Table 2). This confirms that differences in metabolic profiles between children and adults could play a major role on the fatiguing resistance capacity especially when intermittent designs of exercise are used.

Second, the higher muscle mass involved during exercise in adults could account for the observed differences in muscle endurance and fatigability. Higher intramuscular pressure could occur during exercise in adults, thus increasing vascular occlusion and limiting metabolite removal and energy substrate replenishment (Ratel et al., 2008; Kappenstein et al., 2013), potentially leading to lower TTF. These peripheral alterations could interact with psychophysiological mechanisms that may play a fundamental role in determining the premature exercise ending in adults. A higher metabolic by-products accumulation, e.g., H^+ accumulation (Blain et al., 2016), would increase the activation of metabosensitive group III/IV muscle afferents (Amann et al., 2015). These afferents project their input to various sites within the central nervous system (Craig, 1995; Craig, 2003). The central integration of afferent feedbacks [together with the increased corollary discharge, e.g., Amann et al. (2010)] may lead to increased sensations (e.g., effort, pain) involved in exercise termination (Kayser, 2003; Amann et al., 2011). One could speculate that the greater metabolic perturbations in adults would increase these sensations at a higher rate than in children thus leading to a premature exercise ending. These differences in perception of effort [usually reported in these studies into a single "Gestalt" perception, including other sensations like fatigue, discomfort as suggested in the seminal definitions of perceived exertion, e.g., Robertson et al. (1997); Borg (1998)] between children and adults have been addressed in a review (Ratel et al., 2006a), with a common observation that children rate their effort lower than adults (Bar-Or, 1977; Bar-Or, 1989; Ratel et al., 2004; Ratel et al., 2006b), supporting our previous assumption. However, this argument must be balanced with the fact that youth tend to score lower ratings of perceived effort during exercise (Huebner et al., 2014). In the latter study, the median maximum rating for leg exertion in children after cycling was only slightly greater than half the maximum possible value of 10 using the Borg CR10 scale (Borg, 1998). This observation has been confirmed by others (Bar-Or, 1977; Lamb, 1995; Barkley and Roemmich,

2008). This low-rating tendency could be due to the fact that children are unable to correctly understand the scale or properly gauge their perceived exertion, notably because of a lack of previous experiences (Huebner et al., 2014).

The differences in metabolic profile between children and adults could also give some clues to the observed differences in fatigability. The exercise duration has a direct influence on fatigability, with longer exercise inducing greater decrement muscle in performance (Millet, 2011). Then, it would have been incorrect to analyze fatigability data for experimental designs that used exercises of different duration. We thus performed isotime analysis to increase the robustness of our interpretation (Nicolò et al., 2019). For the same exercise duration, it is likely that children prevented the recruitment of high-threshold motor units, partly explaining the lower level of fatigability at isotime. This argument could be valuable for submaximal and maximal exercises if one considers that children have a greater activation deficit that adults. As reported in Table 3, a greater decrement in VA was observed in children after exercise, suggesting that children exhibited more central fatigue than adults. This may be in favor of a specific neural regulation in children during fatiguing exercises that could partly explain our results at isotime. In addition to the common argument of a lower capacity for spatial recruitment in children than in adults, i.e., lesser type-II motor-unit utilization (Dotan et al., 2012), one should also consider differences in temporal recruitment with differences in firing rates of the active motor units between children and adults. Direct evidences for this latter point are lacking so far, and the emergence of novel investigation technique (e.g., high-density electromyography) could help to obtain a more precise overview of differences in motor unit recruitment between children and adults. Last, the low activation level in children could allow the organization of motor units rotation (Ratel and Martin, 2015), which is much more difficult when the level of activation are high.

Further, because of their specific muscle phenotype, adults develop peripheral alterations at a higher rate when compared to children for the same relative exercise duration (see Figure 2 in the following references: Ratel et al. (2015); Bontemps et al. (2019); Piponnier et al. (2019a)) or at exercise termination (see the Pt-related data in Table 3), providing another possibility for the higher level of fatigability recorded at isotime in adults.

While the meta-analyses confirmed that children are 1) less fatigable and 2) able to sustain exercise at a given intensity longer than adults, one could speculate that the role of some specific physiological functioning, e.g., muscle metabolism, involved in the performance would vary as a function of the exercise modality, i.e., type of exercise (isometric versus dynamic) and exercise duration (short versus long-duration exercise), and that it could play a major role in the reported differences in TTF and fatigability between children and adults.

4.2 Differences in TTF and performance fatigability between children and adults depend on the modality of exercise

Sustained or intermittent isometric contractions at a single joint are common to evaluate TTF and fatigability. However, the conclusions derived from these contraction modalities do not necessarily apply for dynamic exercises where the physiological demands and the muscle mass involved in the exercise are different (Enoka and Stuart, 1992; Carroll et al., 2017). Considering the major changes in body size and physiological function over the course of growth and development (Cooper et al., 1987; Cooper et al., 2014), one may expect an influence of the type of exercise on the observed differences in TTF and fatigability between children and adults.

Among the 15 studies that looked at TTF differences between children and adults, 11 used an isometric modality (Armatas et al., 2010; Patikas et al., 2013; Hatzikotoulas et al., 2014; Ratel et al., 2015; Tanina et al., 2017; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Woods et al., 2019; Piponnier et al., 2020; Woods et al., 2020) while four used a dynamic modality that included running and cycling exercises (Berthoin et al., 2003; Leclair et al., 2011; Bar-Yoseph et al., 2019) as well as concentric MVCs (Murphy et al., 2014). We found that children had longer TTF than adults when isometric exercises were performed with no differences for dynamic exercises. Eight out of the 11 isometric studies used intermittent fatiguing protocols. We can speculate that the use of intermittent exercises favored the children in sustaining the exercise for a longer duration, thanks to their higher muscle oxidative activity and their faster regulation of blood acid-base balance. Interestingly, two out of the three studies that used continuous isometric fatiguing protocols showed no differences in TTF between children and adults (Patikas et al., 2013; Tanina et al., 2017). The nature of the exercise (i.e., intermittent versus continuous) could explain the absence of differences between children and adults for the four studies that used dynamic exercises. Indeed, three out of these four studies used continuous exercises and showed either no changes (Leclair et al., 2011) or higher TTF in adults (Berthoin et al., 2003; Bar-Yoseph et al., 2019), while the one that used an intermittent design showed higher TTF in children (Murphy et al., 2014). Overall, these results support the idea that differences in exercise duration between children and adults are more detectable when intermittent exercises are used. One should note, however, the large imbalance in the number of studies included in the quantitative analyses that used isometric (n = 11) versus dynamic (n = 4) exercises which could have prevented detecting possible differences between children and adults for this latter exercise modality.

Among the 19 included studies in fatigability analyses, seven used isometric exercises (Halin et al., 2003; Hatzikotoulas et al., 2009; Ratel et al., 2015; Piponnier et al., 2019a; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020) and 12 used dynamic exercises that included running (Äyrämö et al., 2017),

cycling (Hebestreit et al., 1993; Buchheit et al., 2010; Birat et al., 2018; Weinstein et al., 2018) and jumping (Marginson et al., 2005; Ftikas et al., 2010; Gorianovas et al., 2013; Liamopoulou et al., 2015; Lazaridis et al., 2018) efforts as well as repeated concentric MVCs (De Ste Croix et al., 2009; Dipla et al., 2009). The results revealed that children were less fatigable than adults when performing dynamic rather than isometric exercises. This finding is not consistent with TTF analyses which did not reveal any differences in TTF for dynamic exercises. While any attempt to give a physiological explanation for this result would remain hazardous, one could nevertheless suggest that the use of isotime comparison to compute fatigability in our meta-analysis is a candidate. Isotime data for most children were reported away from the end of the exercise. For instance, isotime comparisons in a study from our group (Piponnier et al., 2019b) were made at the 10th MVC which corresponded to the lowest number of contractions performed by one adult participant while the mean number of contractions was 40 ± 18 (range: 15–79) in children, suggesting that most children were still far from exercise termination. While these two outcomes (i.e., TTF and fatigability) are commonly used interchangeably to inform on exercise-related performance [e.g., Clark et al. (2005)], our results evidenced that they are not. TTF considers the time to exhaustion which rely on physiological mechanisms but also on psychological and motivational ones (Pageaux, 2016). Fatigability, as evaluated in this meta-analysis, informs on what happened on the early-middle phase of the exercise, far from exhaustion for most participants.

We also investigated whether differences in TTF were impacted by the exercise duration. Children are more likely to engage in very short bursts of intense physical activity interspersed with varying intervals of low to moderate intensity (Bailey et al., 1995). This is consistent with our results showing higher between-group differences, favoring children 1) for short compared to long-duration exercises and 2) for intermittent exercises. The classification of short versus long-exercise duration indirectly reflects the exercise intensity that was performed during the exercise. Six out of the seven studies classified in the short-duration category used high or maximal intensity of exercise (Berthoin et al., 2003; Hatzikotoulas et al., 2014; Murphy et al., 2014; Bontemps et al., 2019; Piponnier et al., 2019b; Piponnier et al., 2020) and five out of the seven studies classified in the long-duration category used moderate and submaximal intensities (Leclair et al., 2011; Patikas et al., 2013; Bar-Yoseph et al., 2019; Woods et al., 2019; Woods et al., 2020). Higher oxidative capacities in children could have favored a most effective recovery between intense exercise bouts explaining why they lasted longer than adults. Moreover, the perception of effort reported in children is often lower than in adults for short/intense bouts of exercise (i.e., up to 10 min) (Bar-Or and Rowland, 2004; Ratel et al., 2006a). This is likely due to differences in the way by which peripheral and central signals are integrated (Bar-Or and Rowland, 2004). Then, the perception of effort may increase at a higher rate in adults for short-duration and high-intensity exercises, contributing to earlier exercise termination, possibly explaining why children performed better for short-than long-duration exercises. Finally, differences in thermoregulation processes have been reported between children and adults (Notley et al. (2020) for a review). For instance, children present a lower rate of sweating than adults that is known to have a negative influence on body temperature regulation during prolonged exercise. This could lead to a higher metabolic demand relative to body mass in children, thus lowering the exercise economy (Rowland, 2008) and impacting exercise performance for long-duration exercises, especially involving whole-body tasks.

Overall, both the type of exercise and exercise duration can modulate the reported differences in fatigability and TTF between children and adults. Beyond these two modalities, it seems that the intermittent versus continuous design of exercise plays a significant role in these differences.

4.3 Physiological changes during maturation influence the exercise-related performance

Because significant changes occur in physiological systems during the transition from childhood to adulthood, there is a necessity to make a clear distinction between children and adolescents in the literature to have a precise overview of their potential differences in exercise-related performance especially when compared to adults. To apprehend the maturation-related influence on exercise performance in the best possible way, it would have been interesting to report children *versus* adolescents comparisons in dedicated meta-analysis. It was, however, not the primary purpose of this systematic review so as the inclusion criteria were not chosen in that way. Actually, only four and three studies could have been included in meta-analysis comparing children and adolescents for potential differences in fatigability and TTF, respectively, which is obviously too low to provide any robust and interpretable data. However, and when possible and relevant, children *versus* adolescents differences are descriptively discussed in the following paragraphs.

Differences in TTF and fatigability between adolescents and adults were investigated in only five (Pullinen et al., 2002; Pullinen et al., 2011; Tibana et al., 2012; Piponnier et al., 2019a; Bar-Yoseph et al., 2019) and seven (Kanehisa et al., 1995; Pullinen et al., 2002; Dipla et al., 2009; Buchheit et al., 2010; Willcocks et al., 2014; Äyrämö et al., 2017; Piponnier et al., 2019a) studies, respectively. Our analysis showed a longer but not significant ($p = 0.09$) TTF in adolescents together with a lower level of fatigability, when compared to adults. Only few studies compared children, adolescents and adults within the same experimental design. The results regarding TTF are controversial. Some authors reported longer TTF in children and adolescents when compared to adults, and a trend ($p = 0.05$) for longer TTF in children than adolescents (Piponnier et al., 2019a). Others reported longer TTF in adolescents

and adults than children during running exercises (with no differences during cycling) (Bar-Yoseph et al., 2019). Eight studies investigated differences in fatigability between the three populations, some of them being not included in the statistical analyses because of methodological concerns (Ratel et al., 2002; Chen et al., 2014). Overall, adolescents are more fatigable than children (Ratel et al., 2002; Faigenbaum et al., 2008; Dipla et al., 2009; Chen et al., 2014; Piponnier et al., 2019a) but less than adults.

This confirms that growth and maturation influence the level of fatigability likely because of specific neuromuscular changes that are attributed to the puberty (Ratel and Martin, 2015). First, adolescents engage a higher muscle mass during the exercise (Van Praagh and Doré, 2002). This could be the origin of greater metabolic perturbations, especially because of greater intramuscular pressure during exercise. Differences in muscle typology [i.e., higher proportion of type II muscle fibers in adolescents, e.g., Glenmark et al. (1994)] or in energy metabolism [i.e., lower oxidative activity for ATP synthesis in adolescents, e.g., Berg and Keul (1988)] contribute to the higher level of fatigability observed in adolescents. Second, adaptations within the central nervous system during the maturation process could contribute to these differences. Children are less able to voluntarily recruit their motor units during exercise, likely due to an immaturity of the corticospinal pathway (Nezua et al., 1997). Besides this lower recruitment capacity, they would recruit a higher relative proportion of slow-twitch fibers and would benefit from a more organized and efficient motor unit rotation (Ratel and Martin, 2015). Because of the limited number of studies that investigated how peripheral and central modulations could differentially impact the exercise-related performance between children and adolescents, these arguments remain speculative. Only two studies investigated in the same experimental design the neural and peripheral functioning in response to exercise in children, adolescents and adults. Our group recently reported that Pt amplitude was reduced in adolescents after an intermittent isometric exercise while it was not in children (Piponnier et al., 2019a), suggesting that contractile properties and/or excitation-contraction coupling was preserved in children while altered in adolescents (Table 3). In contrast, VA decreased at a similar level after exercise in children and adolescents, while it remained unchanged in adults, suggesting that the greater central fatigue in children and adolescents likely account for their lower degree of peripheral alterations than adults. These isolated results strengthen the hypothesis of an evolution in the maturation of the central nervous system during growth, with the tolerance of the central nervous system to peripheral alterations increasing during puberty (Hamada et al., 2003). Besides this pioneer theory claiming that peripheral functioning is preserved by central regulation in children, one should also consider that the explanation could directly come from the muscle functioning, i.e., the fatigue-resistant muscles of the children do not develop a large amount of fatigue independently of any central influence. Another study investigated the peripheral and central factors of fatigue after a 50-s maximal run (Äyrämö et al., 2017) and confirmed the aforementioned observations. Of note, a large delay (i.e., 6–12 min) separated the end of the exercise and the beginning of neuromuscular testing in the latter study (Äyrämö et al., 2017). Considering the rapid recovery of neuromuscular function that occurs within the first 2-min after exercise (Froyd et al., 2013; Gruet et al., 2014; Mira et al., 2017), these latest results should be interpreted with caution.

4.4 Limitations

Some limitations pertaining to our analyses must be acknowledged. First, most outcomes displayed a moderate to high level of heterogeneity, likely due to differences in experimental procedures, e.g., type of exercise (isometric versus dynamic, long versus short duration, intermittent versus continuous), muscles involved (e.g., lower versus upper limbs) and population characteristics. These high levels of heterogeneity are, however, common in this kind of quantitative analyses, e.g., Kruger et al. (2018). A step towards a standardization of experimental protocols to evaluate TTF and fatigability in children, adolescents and adults could help in making the results of individual studies more homogeneous to establish more robust conclusions. Second, the overall quality of the studies included in the meta-analysis was low (mean quality score of 4.3 ± 1.2 stars out of a maximum of 9 stars) which could have led to a biased estimation of the between-group differences in TTF and fatigability. For instance, the overall score of the comparability domain was low (e.g., 0.9 ± 0.7 stars out of a maximum of 2 stars) meaning that physical activity status in some studies was not rigorously controlled. This is an important observation because the level of physical activity influences TTF and fatigability (Murphy and Smith, 2010; Buchowski et al., 2013) and varies during childhood (Guinhouya et al., 2013). Most of the included studies monitored the level of physical activity by the mean of self-report that involved questionnaires or brief interviews. These subjective measures can misjudge the absolute level of physical activity (Prince et al., 2008). Objective measures (e.g., accelerometers) can increase the precision of this important outcome. Only a minor proportion (~12%) of the included studies used objective measures to capture the level of physical activity (Hebestreit et al., 1993; Buchheit et al., 2010; Weinstein et al., 2018; Bar-Yoseph et al., 2019), and one of them did not match the participants for similar level of physical activity (Buchheit et al., 2010). Third, a risk of publication bias was suggested for fatigability indicating that our analyses could overestimate fatigability differences between children and adults. Last, we excluded some studies because of the lack of information [e.g., absence of standard deviation (Gaul et al., 1995; Ratel et al., 2002), no access to the Pre-versus Post-exercise relative changes (Chen et al., 2014), no access to the contraction duration during intermittent exercises (Soares et al., 1996; Faigenbaum et al., 2008)], and their inclusion could have slightly influenced the results obtained in the meta-analyses.

5 Perspectives

Children are able to sustain an exercise longer and are less fatigable than adults. The selected exercise modality, i.e., the type and the duration of the exercise, in addition with fundamental differences in physiological functioning influences the reported differences between children and adults. While differences may also exist between children and adolescents, the low number of studies so far prevent a robust interpretation. Further studies using novel experimental techniques (e.g., transcranial magnetic stimulation, high-density electromyography) should be considered to gain novel insights into the interplay that could exist between peripheral and central mechanisms throughout maturation and that could explain the reported differences between children, adolescents and adults. Here are some practical recommendations that could increase the robustness of data interpretation when TTF and fatigability are evaluated in children, adolescents and adults:

1) Monitoring the level of physical activity by objective measures and pairing participants according to this outcome.
2) Performing isotime analysis when fatigability is assessed.
3) Recording both TTF and fatigability to obtain complementary information on exercise performance.
4) Combining various methods to assess the specific role of the central nervous system in children' fatigability.
5) Combining different exercise modalities within the same study to capture the whole facet of the differences in TTF and fatigability.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

RS and MG: Review idea, Conception of article, Literature search, Data analysis, Writing- Original draft preparation, Reviewing and Editing. MC: Data analysis, Writing, Reviewing and Editing. VM, EP, and PD: Writing, Reviewing and Editing.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1026012/full#supplementary-material

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Resting and exercise metabolic characteristics in obese children with insulin resistance

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Purpose: This study aimed to explore the characteristics of resting energy expenditure (REE) and lipid metabolism during incremental load exercise in obese children and adolescents with insulin resistance (IR) to provide evidence for exercise intervention in obese children and adolescents with IR.

Method: From July 2019 to August 2021, 195 obese children and adolescents aged 13–17 were recruited through a summer camp. The participants were divided into IR (n = 67) and no-IR (without insulin resistance, n = 128) groups and underwent morphology, blood indicators, body composition, and resting energy consumption gas metabolism tests. Thirty participants each were randomly selected from the IR and no-IR groups to carry out the incremental treadmill test.

Results: Significant metabolic differences in resting and exercise duration were found between the IR and no-IR groups. In the resting state, the resting metabolic equivalents (4.33 ± 0.94 ml/min/kg vs. 3.91 ± 0.73 ml/min/kg, p = 0.001) and REE (2464.03 ± 462.29 kcal/d vs. 2143.88 ± 380.07 kcal/d, $p < 0.001$) in the IR group were significantly higher than in the no-IR group. During exercise, the absolute maximal fat oxidation (0.33 ± 0.07 g/min vs. 0.36 ± 0.09 g/min, p = 0.002) in the IR group was significantly lower than in the no-IR group; maximal fat oxidation intensity (130.9 ± 8.9 bpm vs. 139.9 ± 7.4 bpm, p = 0.040) was significantly lower in the IR group.

Conclusion: Significant resting and exercise metabolic differences were found between obese IR and no-IR children and adolescents. Obese IR children and adolescents have higher REE and lower maximal fat oxidation intensity than obese no-IR children and adolescents.

KEYWORDS

insulin resistance, obesity, children/adolescents, FATmax, fat metabolic flexibility

Introduction

The prevalence of obesity in children and adolescents has become a global public health problem (Collaborators et al., 2017), and the incidence of obesity-related metabolic diseases, such as cardiovascular disease, type 2 diabetes mellitus, and insulin resistance (IR), has increased annually (Gastaldelli et al., 2007; Neeland et al., 2012; Neeland et al., 2013). Studies revealed that the prevalence of IR among obese children and adolescents was 44.3%, which is much higher than the prevalence in overweight (28.6%) and normal weight (8.9%) children and adolescents (Yin et al., 2013). In the past studies, IR refers to an abnormal state of decreased insulin sensitivity in body tissues, such as the liver or muscle. It was not only the pathophysiological basis of many metabolic diseases (Reaven, 1988; Lee, 2006), but also an important risk factor for cardiovascular diseases (Adeva-Andany et al., 2019). IR in children and adolescents also leads to an increased incidence of cardiovascular disease in adulthood (Yajnik et al., 2015). IR was usually accompanied by beta-cell decompensation, which was more rapid in children and adolescents than in adults and more likely to result in complications (Copeland et al., 2011).

Researchers have suggested that the high incidence of obesity-induced IR may be closely related to abnormalities in glucolipid metabolism, lipid accumulation, and mitochondrial dysfunction (Befroy et al., 2007; Boudina and Graham, 2014; Girousse et al., 2018). As a metabolic disease induced by obesity, IR causes population undergone skeletal muscle mitochondrial dysfunction with reduced ATP synthesis capacity and impaired lipid oxidation (Stump et al., 2003), (i.e., impaired energy substrate conversion), which is central to the pathophysiology associated with IR (Galgani et al., 2008). Current studies on functional substrate related research in IR populations were mostly based on positive energy balance (i.e., energy intake exceeds energy expenditure, like oral glucose tolerance test or high-fat diet intervention), while there were fewer studies related to functional characteristics of substrates in negative energy balance (i.e., energy expenditure exceeds energy intake, through exercise, for instance).

Exercise can be effective in improving IR (Marson et al., 2016; Lee et al., 2019), and exercise intensity is an important factor in the effectiveness of interventions. As far as we know, there are few studies related to resting energy expenditure (REE) in obese IR children and adolescents, which has led to the lack of corresponding diagnostic criteria for exercise intensity in this population. REE is an essential component of daily energy needs and accounts for approximately 60%–70% of total energy expenditure in resting state (Johnstone et al., 2005), and recognized as deriving from biochemical reactions at subcellular and cellular levels. During exercise, as exercise intensity increases, the rate of fat oxidation reaches a maximum at a certain intensity (Brooks and Mercier, 1985), at which time the exercise intensity reaches maximal fat oxidation intensity (FATmax). Theoretically the maximum amount of fat can be consumed when exercising at FATmax (Jiang et al., 2020). Fat accumulation was a major causative factor for IR, and exercise intervention with FATmax to improve IR is well worth exploring. However, few studies have been conducted on the exercise lipid metabolic characteristics of obese children and adolescents with IR, so investigating resting and exercise metabolic characteristics can provide a theoretical reference for IR treatment.

We hypothesized that obese IR children and adolescents have higher REE and lower FATmax than obese no-IR children and adolescents. In this study, we investigated the basal metabolic levels of obese IR children and adolescents. We also investigated the FATmax levels of obese IR children and adolescents during the incremental treadmill test and the lipid metabolism characteristics at different exercise intensities to provide theoretical guidance for exercise intervention for this population.

Participants and methods

Participants

This study was conducted with obese children and adolescents who participated in a summer camp in South China (Huizhou, China) between July 2019 and August 2021. The screening criteria were as follows (Collaborators et al., 2017): age between 13 and 17 years (Neeland et al., 2012); the diagnosis of obesity according to the Chinese standard, which was developed based on Chinese children and adolescents (GoCOT, 2004); and (Neeland et al., 2013) able to cooperate with the researchers to complete relevant tests. The exclusion criteria were as follows (Collaborators et al., 2017): morbidly obese children and adolescents with severe obesity complications (≥40 kg/m^2; confirmed cardiovascular disease, obstructive sleep apnoea, musculoskeletal problems, or idiopathic intracranial hypertension) (Neeland et al., 2012); unable to complete the exercise test (Neeland et al., 2013); taking medication for obesity or other diseases; and (Gastaldelli et al., 2007) fasting plasm glucose (FPG) > 5.60 mmol/L. All the subjects and their parents were informed of the benefits and possible risks associated with the experiment and signed an informed consent form before the experiment. The subjects finished the self-reported pubertal development scale, which can effectively evaluate the puberty development stage of Chinese children and adolescents (Zhu and Chen, 2012). This study was approved by the Ethics Committee of the Guangzhou Sports Institute (Approval number: 2018LCLL-008).

A total of 195 eligible subjects were enrolled in this study, underwent basic information collection, and completed the resting energy test. Thirty participants each were randomly selected from the IR and no-IR groups for the exercise energy

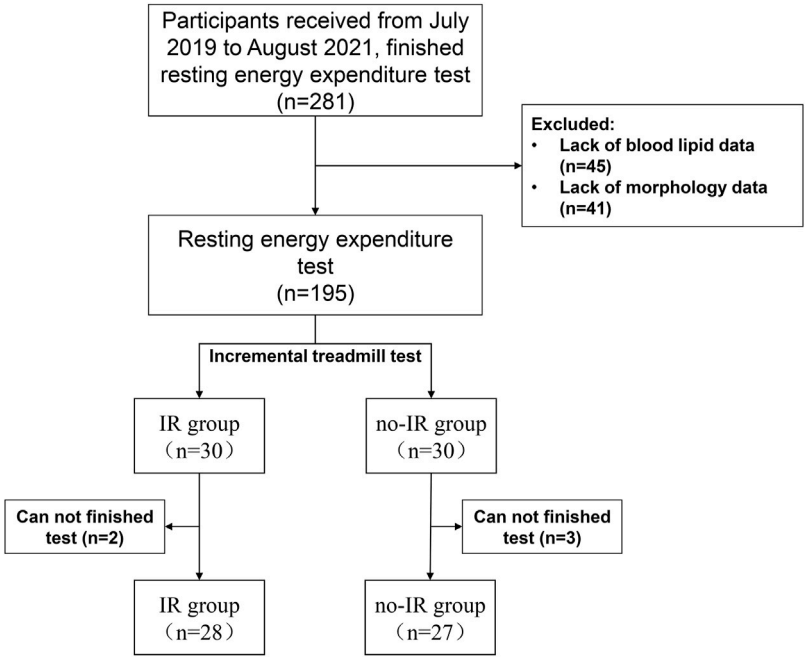

FIGURE 1
Participants screened flow.

test. A tatal of 28 participants in the IR group and 27 in the no-IR group finished the test (Figure 1).

Morphology and blood indices

Height, body weight (BW), waist circumference (WC), hip circumference (HC), and body composition were measured in the morning after fasting overnight. Height was measured to the nearest 0.1 cm using a standard height meter, and the BW was measured to the nearest 0.1 kg on a digital scale. Body mass index (BMI) was calculated by weight in kilograms divided by the square height in meters. WC and HC were measured using an inelastic plastic fibre tape measure placed directly on the skin at the midpoint between the lower border of the rib cage and the iliac crest (WC) (Autonomous, 1995) and at the maximum extension of the buttocks (HC) (Wang et al., 2000). The participants wore short sleeves and shorts and were not allowed to wear any metal accessories. Body composition was measured using a body composition meter (Inbody, 370, Korea). The subjects kept quiet until the end of the test.

Fasting venous blood samples were collected in the early morning, and the upper layer of the plasma was extracted after low-temperature centrifugation and stored in a refrigerator at −80°C for testing. FPG was measured using the glucose oxidase method, and fasting insulin (FINs) was measured through the electrochemiluminescence method, and total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were tested and analysed using a fully automated biochemical analyser (AU5800, Beckman, Japan). The homeostasis model assessment index of insulin resistance (HOMA-IR) was calculated according to Formula (Collaborators et al., 2017) for the differential diagnosis of IR, and the criterion was HOMA-IR > 3 (Yin et al., 2013).

$$HOMA-IR=\frac{FINs\left(\frac{\mu U}{L}\right)*FPG\left(\frac{mmol}{L}\right)}{22.5} \quad (1)$$

Indirect calorimetry measurement

REE and incremental load exercise energy consumption were measured using indirect calorimetry, which is the gold standard for energy metabolism measurements (Boullata et al., 2007). Energy consumption tests were performed using a portable gas metabolic meter (Cortex Meta Max 3B, CORTEX, Germany). After warming up the instrument, the ambient gas (before each test), barometric pressure (once a day), and volume (once a day) were set, and standard gas calibration was performed once a month. The standard gases are 15% O_2, 5% CO_2, and 80% N_2 (CORTEX, Germany).

The REE test was performed in the supine position in the morning under a fasted state. All the participants were kept awake and quiet during the test, and physical activities were prohibited. The room temperature was 25–27°C, and the participants' carbon dioxide production (VCO_2) and oxygen consumption (VO_2) were recorded continuously for 15 min.

The FATmax test was performed using an incremental load treadmill test. Given that the recommended minimum phase duration to reach a steady metabolic state for obese children and adolescents is no less than 3 min (Bordenave et al., 2007; Brun et al., 2011; Chrzanowski-Smith et al., 2018), the exercise test time for each phase was 5 min. The initial speed of the incremental load was 3 km/h, followed by 1 km/h stepwise increments, to 4 km/h, 5 km/h, 6 km/h, and 7 km/h, respectively. The termination criterion was respiratory exchange rate (RER) > 1.0 (Macfarlane, 2017), or exhaustion. During the test, the participants wore a heart rate monitor (H7, Polar, Finland) to monitor their heart rate (HR) in real time.

Calculation

The first 1–10 min was the adaptation period, and 11–15 min was the recorded average to calculate VCO_2 and VO_2. The adaptation period was 1–3 min for each level of the incremental treadmill test, and the average VCO_2 and VO_2 at each level were calculated by intercepting 4–5 min of data. The fat oxidation levels were calculated according to Frayn (Eqs 2, 3) (Frayn, 1983). For each subject, the calculated values of fat oxidation were depicted graphically as a function of exercise intensity, and a third polynomial fitting curve with the intersection in (0,0) was constructed to determine the relative exercise intensities that elicited FATmax and the maximal fat oxidation rate (FOmax, g/min) (Achten et al., 2002). Metabolic flexibility was defined as the difference between the FOmax and the resting fat oxidation. REE was calculated using the Weir formula (Eq. 4) (Weir, 1949), and the RER was calculated from VCO_2 and VO_2 (Eq. 5).

$$FO = 1.67 \times VO2 - 1.67 \times VCO2 \quad (2)$$

$$CHO = 4.55 \times VCO2 - 3.21 \times VO2 \quad (3)$$

$$REE = 3.941 \times VO2\,(L/min) + 1.11 \times VCO2\,(L/min) \quad (4)$$

$$RER = \frac{VCO2\,(L/min)}{VO2\,(L/min)} \quad (5)$$

Statistical analysis

GraphPad Prism 8 (GraphPad Prism Software Inc., San Diego, CA) was used to draw violin plots and line graphs. Statistical package for social sciences (SPSS, version 24, IBM Corporation, United States) software was used for the statistical analysis of the data. Continuous variables were tested for normality using the Kolmogorov–Smirnov method, and the data conforming to a normal distribution are expressed as mean ± standard deviation (mean ± SD). One-way ANOVA was used to analyze the differences between groups. The data that did not follow a normal distribution were expressed as median (quartiles) [M (P25, P75)], and the Mann–Whitney U test was used. Pearson correlation was used to determine the relationships between REE and fat free mass (FFM). For all the statistical analyses, significance was accepted at $p < 0.05$. Categorical variables were compared between group differences using chi-square tests. The relevant influence factors of REE were explored by multiple linear regression.

Results

Participants characteristics

A total of 195 eligible participants were included in this study and divided into the IR group ($n = 67$) and the no-IR group ($n = 128$); the prevalence of IR was 34.36%. BW, WC, HC, body fat (BF), body fat percentage (BFP), TC, and TG were significantly higher in the IR group than in the no-IR group ($p < 0.05$), whereas there was no significant difference between the FFM, FPG, LDL-c, and HDL-c of the IR and no-IR groups ($p > 0.05$). Among the IR group, 90.3% were in late adolescence or post-puberty, whereas 92.1% of the no-IR group were in this stage (Table 1).

Resting metabolic test results

The resting metabolism test results showed a significant difference between the IR and no-IR groups. The metabolic equivalent (MET) (4.33 ml/min/kg vs. 3.91 ± 0.73 ml/min/kg, $p = 0.001$) and REE (2464.03 ± 462.29 kcal/d vs. 2143.88 ± 380.07 kcal/d, $p < 0.001$) were significantly higher in the IR group than in the no-IR group. The proportion of lipid energy supply at rest was significantly lower in the IR group than in the no-IR group (0.55 ± 0.20 vs. 0.62 ± 0.20, $p = 0.033$). REE was highly correlated with FFM in the IR group (r = 0.803, $p < 0.001$) and no-IR group (r = 0.592, $p < 0.001$), and the correction between FFM and REE was higher in the IR group than in the no-IR group. When REE was normalized for FFM, REE/FFM was found to be significantly higher in the IR group than in the no-IR group ($p = 0.002$). BF and BFP were significantly higher in the IR group than in the no-IR group ($p < 0.05$); after normalized for BF, the results show no significant difference between REE/BF of the IR and no-IR groups ($p = 0.076$) (Table 2).

TABLE 1 Baseline characteristics of the participants.

	Total (n = 195)	IR Group (n = 67)	No-IR Group (n = 128)	p-value
Age	14.01 ± 1.00	14.12 ± 1.07	13.95 ± 0.97	0.312
Male/Female	102/93	38/29	64/64	0.373
Height (cm)	165.37 ± 7.94	166.86 ± 7.58	164.59 ± 8.05	0.057
BW (kg)	82.13 ± 12.35	84.94 ± 12.74	80.67 ± 11.94	0.022
BMI	29.99 ± 3.55	30.49 ± 3.47	29.73 ± 3.58	0.160
WC (cm)	99.69 ± 10.05	101.67 ± 10.24	98.66 ± 9.84	0.047
HC (cm)	106.90 ± 7.75	109.04 ± 7.78	105.79 ± 7.52	0.005
BFP	33.26 ± 5.89	34.57 ± 6.68	32.58 ± 5.33	0.024
FFM (kg)	53.71 ± 8.64	54.90 ± 9.60	53.07 ± 8.09	0.161
BF (kg)	28.40 ± 8.17	30.03 ± 8.58	27.60 ± 7.79	0.047
FINs (μU/L)	12.87 ± 10.32	21.98 ± 12.48	8.10 ± 3.88	< 0.001
FPG (mmol/L)	4.91 ± 0.96	4.97 ± 0.85	4.88 ± 1.02	0.569
TC (mmol/L)	4.26 ± 1.33	4.58 ± 1.80	4.09 ± 0.93	0.014
HDL-c (mmol/L)	1.13 ± 0.23	1.11 ± 0.21	1.14 ± 0.23	0.389
TG (mmol/L)	1.02 ± 0.54	1.19 ± 0.58	0.93 ± 0.50	0.002
LDL-c (mmol/L)	2.38 ± 0.74	2.48 ± 0.75	2.33 ± 0.73	0.192
HOMA-IR	2.76 ± 2.37	4.80 ± 3.01	1.69 ± 0.71	< 0.001
Stages of puberty	143	41	102	
Mid-Pubertal	12 (8.4%)	4 (9.7%)	8 (7.9%)	
Late Puberty	28 (19.6)	11 (26.8%)	17 (16.7%)	
Postpubertal	103 (72.0%)	26 (63.4%)	77 (75.5%)	

BMI, body mass index; BF, body fat; BFP, body fat percent; BW, body weight; FFM, Fat Free Mass; FPG, fasting plasma glucose; FINs, fasting insulin; HOMA-IR, Homeostasis Model Assessment index of Insulin Resistance; HC, hip circumference; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TC, Total cholesterol; TG, Triglycerides; WC, waist circumference.

TABLE 2 Characteristics of resting metabolic in IR and no-IR group.

	Total (n = 195)	IR group (n = 67)	No-IR group (n = 128)	p-value
VO2 (ml/min)	330.54 ± 63.16	359.58 ± 66.93	315.35 ± 55.58	< 0.001
VCO2 (ml/min)	250.98 ± 58.18	280.71 ± 61.18	235.42 ± 50.16	< 0.001
1MET (ml/min/kg)	4.06 ± 0.83	4.33 ± 0.94	3.91 ± 0.73	0.001
RER	0.77 ± 0.05	0.78 ± 0.04	0.77 ± 0.04	0.021
REE (kcal/d)	2253.88 ± 436.47	2464.03 ± 462.29	2143.88 ± 380.07	< 0.001
REE/BW (kcal/d/kg)	27.60 ± 4.51	29.17 ± 4.62	26.77 ± 4.24	< 0.001
REE/FFM (kcal/d/kg)	42.50 ± 9.98	46.59 ± 12.76	40.95 ± 8.08	0.002
REE/BF (kcal/d/kg)	84.95 ± 27.72	89.81 ± 34.72	82.41 ± 22.99	0.076
FO (g/min)	0.12 ± 0.03	0.13 ± 0.04	0.12 ± 0.03	0.024
CHO (g/min)	0.10 ± 0.07	0.12 ± 0.07	0.09 ± 0.07	0.002
Proportion of FO	0.60 ± 0.20	0.55 ± 0.20	0.62 ± 0.20	0.033

BF, Body Fat; BW, Body Weight; CHO, Carbohydrate Oxidation; FO, Fat Oxidation; FFM, Fat Free Mass; MET, Metabolic Equivalent; Ratio of FO, Proportion of fat oxidation; REE, Resting Energy Expenditure; RER, Respiratory Exchange Rate; VCO_2, Carbon Dioxide Production; VO_2, Oxygen Consumption.

Multiple linear regression analysis of resting energy expenditure

Multiple linear regression analysis was performed using REE as the dependent variable and gender, age, pubertal grade, FFM, BF, and HOMA-IR as independent variables (Table 3). The results revealed that REE was significantly correlated with FFM and HOMA-IR levels ($p < 0.05$), but not with gender, age, pubertal stage, and BF ($p > 0.05$).

TABLE 3 Multiple linear regression analysis of resting energy expenditure.

Independent variable	β-coefficient	*p*-value	Model R^2	Model *p*-value
Gender	126.894	0.185	0.249	< 0.001
Age	18.522	0.513		
Puberty	−90.181	0.084		
FFM	11.198	0.047		
BF	−1.041	0.841		
HOMA-IR	55.452	< 0.001		

BF, Body Fat; FFM, Fat Free Mass; HOMA-IR, Homeostasis Model Assessment index of Insulin Resistance.

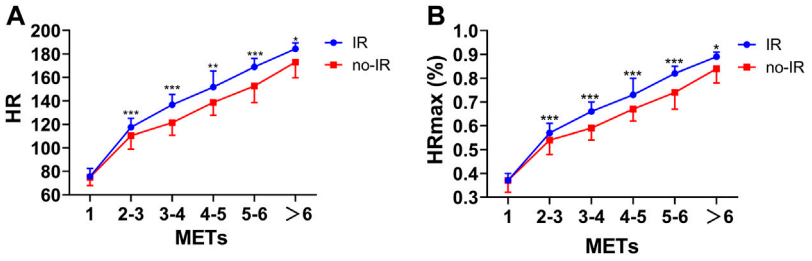

FIGURE 2
HR and HRmax (%) response to the incremental treadmill test in the IR group and no-IR group. Line graph for theComparsion of HR response in different METs. **(A)**: Comparsion of HR response to incremental treadmill test in the IR group and no-IR group; **(B)**: Comparsion of HRmax (%) response to incremental treadmill test in the IR group and no-IR group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ between IR and no-IR groups.

Heart rate response to the incremental treadmill test

During the test, HR gradually increased with intensity, and HR response was significantly higher in the IR group than in the no-IR group. Under the same intensity, HR and HRmax% were significantly higher in the IR group than in the no-IR group ($p < 0.05$) (Figure 2).

Fat and carbohydrate metabolic response to the incremental treadmill test

The incremental treadmill test revealed that the absolute fat oxidation (AFO) increased as with the intensity in both the IR and no-IR groups. The absolute fat oxidation of the IR and no-IR groups decreased with increasing intensity when the exercise intensity exceeded 4 and 5 METs, respectively. Further analysis of the relative fat oxidation (RFO) of the IR and no-IR groups revealed that the RFO of the IR group was significantly lower than that of the no-IR group when the exercise intensity exceeded 3 METs ($p < 0.05$). In addition, the absolute carbohydrate oxidation energy supply level and the relative carbohydrate oxidation were also significantly higher in the IR group than in the no-IR group when the exercise intensity exceeded 3 METs ($p < 0.05$ and $p < 0.001$, respectively). When the exercise intensity exceeded 3 METs, the proportion of lipid energy supply was significantly lower in the IR group than in the no-IR group at the same exercise intensity ($p < 0.01$). The carbohydrate energy supply in the IR group was significantly higher than in the no-IR group when the exercise intensity exceeded 3 METs. The proportion of fat energy supply was lower in the IR group than in the no-IR group when the exercise intensity exceeded 3 METs during exercise (Figure 3).

Fat oxidation and FATmax in insulin resistance and no-insulin resistance groups

Calculations attained by fitting a third order polynomial fit curve for fat oxidation kinetics for each participant revealed that the absolute maximal fat oxidation was significantly lower in the IR group than in the no-IR group (0.33 ± 0.07 g/min vs. 0.36 ± 0.09 g/min, $p = 0.002$) The relative maximal fat oxidation was also lower in the IR group than in the no-IR group (3.93 ± 0.89 mg/min/kg vs. 4.49 ± 1.00 mg/min/kg, $p = 0.038$). When the exercise intensity reached FATmax, the IR group had significantly lower HR values (130.9 ± 8.9 bpm vs. 139.9 ± 7.4 bpm, $p = 0.040$) and a lower HRmax% than the no-IR group (0.63 ± 0.04 vs. 0.68 ± 0.03, $p = 0.039$); the METs of the IR and no-IR groups showed no significant difference (3.50 ± 0.71 vs. 4.19 ± 0.86, $p = 0.158$) (Figure 4).

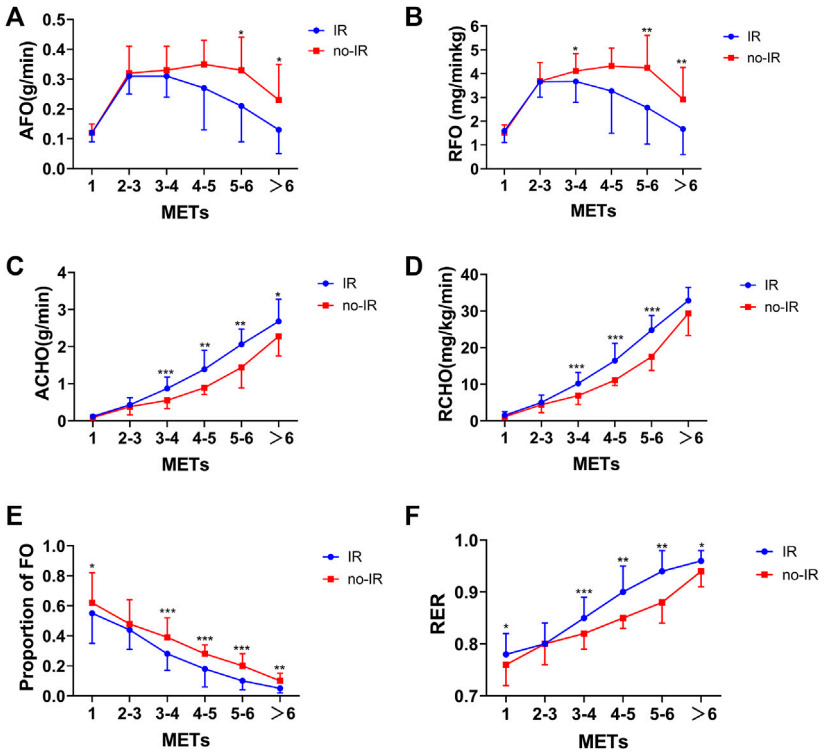

FIGURE 3
Fat and carbohydrate oxidation response to incremental treadmill test in IR and no-IR groups. Line graph of metabolic characteristics at different intensities. **(A)**: AFO response to incremental treadmill test in IR and no-IR groups; **(B)**: RFO response to incremental treadmill test in IR and no-IR groups; **(C)**: Absolute carbohydrate oxidation (ACHO) response to incremental treadmill test in IR and no-IR groups; **(D)**: Relative carbohydrate oxidation (RCHO) response to incremental treadmill test in IR and no-IR groups; **(E)**: Proportion of FO during incremental treadmill test; **(F)**: RER changes during incremental treadmill test in IR and no-IR groups. *P < 0.05, **P < 0.01, ***P < 0.001 between IR and no-IR groups.

Fat metabolic flexibility in insulin resistance and no-insulin resistance groups

Significant differences in fat metabolic flexibility were observed during the incremental treadmill test. Absolute fat metabolic flexibility was significantly lower in the IR group than in the no-IR group (0.19 ± 0.06 g/min vs. 0.25 ± 0.09 g/min, $p = 0.010$); after adjusting the body weight factors, relative fat metabolic flexibility remained significantly lower in the IR group than in the no-IR group (2.34 ± 0.80 mg/min/kg vs. 3.20 ± 0.87 mg/min/kg, $p = 0.001$) (Figure 5).

Discussion

The main findings of this study are as follows (Collaborators et al., 2017): The energy metabolism characteristics of the IR and no-IR groups have a significant difference. The 1 MET and REE in the IR group were significantly higher than in the no-IR group, and the proportion of fat supply was significantly lower in the IR group (Neeland et al., 2012). The absolute maximal fat oxidation rate was significantly lower in the IR group, and the FATmax was also smaller in the IR group (Neeland et al., 2013). During the incremental treadmill test, the rate of fat oxidation in the no-IR group was significantly higher than in the IR group at the same exercise intensity, whereas the HR response was milder (Gastaldelli et al., 2007). The fat metabolic flexibility was significantly lower, and the level of fat mobilization was weaker in the IR group than in the no-IR group.

The diagnosis of obesity according to the Chinese standard. Because the participants were Chinese, the obesity identification criteria were especially developed for Chinese, which were also in consistent with the eastern Asia ethnic characteristics (GoCOT, 2004). Two international references are widely used: the International Obesity Task Force reference (Cole et al., 2000) and the World Health Organization standard 2007 (de Onis et al., 2007), but the two references applied to the same children lead to different obesity prevalence rates (Wang and Wang, 2002).

The REE in the IR group was significantly higher than in the no-IR group, which was generally consistent with previous studies (Ten et al., 2008). Some studies found that the main

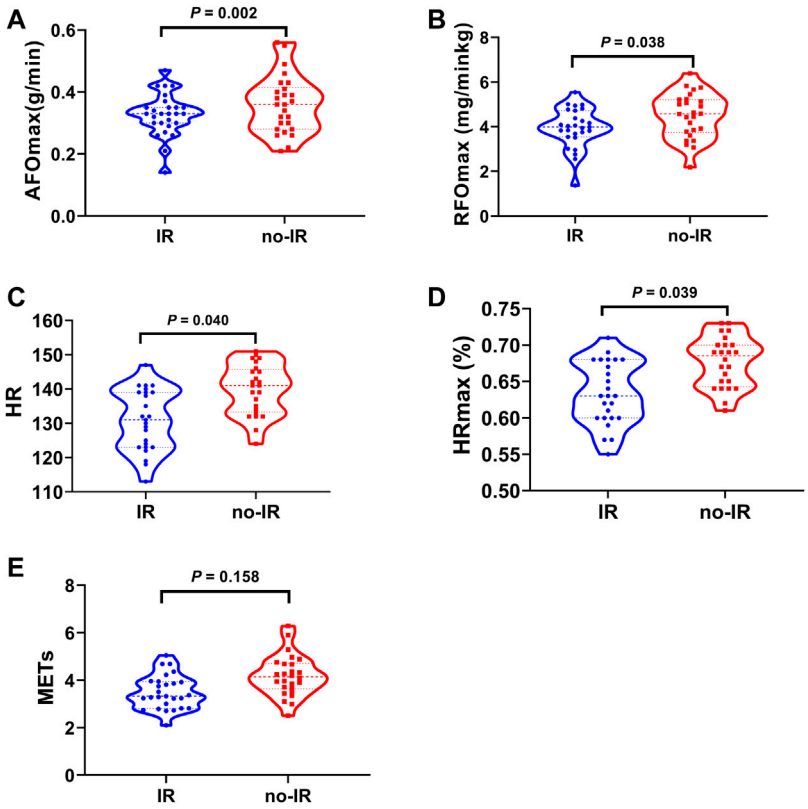

FIGURE 4
Fat Oxidation and FATmax in IR and no-IR groups. Violin plot of fat oxidation characteristics between IR and no-IR group. **(A)**: Absolute maximal fat oxidation rate (AFOmax) between IR and no-IR groups; **(B)**: Relative maximal fat oxidation rate (RFOmax) between IR and no-IR groups; **(C)**: FATmax (HR) between IR and no-IR groups; **(D)**: FATmax (HRmax %) between IR and no-IR groups; **(E)**: FATmax (METs) between IR and no-IR groups.

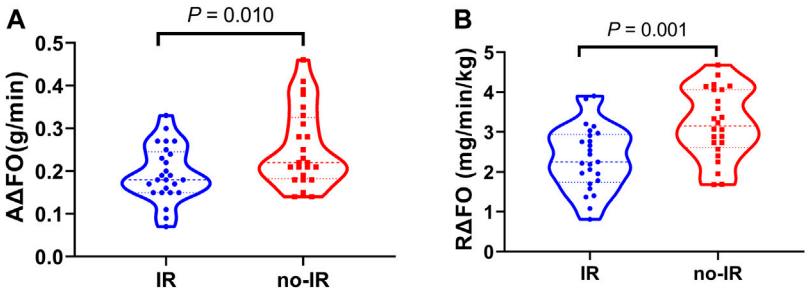

FIGURE 5
Fat metabolic flexibility between IR and no-IR groups. Violin plot of fat metabolic flexibility between IR and no-IR group. **(A)**: Absolute fat metabolic flexibility (AΔFO) between IR and no-IR groups; **(B)**: Relative fat metabolic flexibility (RΔFO) between IR and no-IR groups.

influence factors of REE were BW, FFM and metabolic status (Bandini et al., 1990; Goran and Treuth, 2001; Ten et al., 2008). After conducting multiple linear analyses, we found that FFM and HOMA-IR levels were important determinants of REE. Although, the FFM in the IR and no-IR groups did not differ significantly in this study (54.90 ± 9.60 kg vs. 53.07 ± 8.09 kg, $p = 0.161$). The BF in the IR group was significantly higher than in the no-IR group ($p < 0.05$), the main reason may be that FFM

(50% of skeletal muscle) has a greater impact on REE than BF (Molnár and Schutz, 1997). FFM consists of highly metabolically active muscle and organs, such as the skeletal muscle's metabolic rate was 13 kcal/kg/d, and thus the average 25 kg organ was predicted to consume 325 kcal/d or roughly 13% of REE (Heymsfield et al., 2021). Another important factor affecting REE was HOMA-IR. The degree of IR showed a significant positive correlation with REE. This may be a consequence of an increased neoglucogenetic activity. Neoglucogenesis is, an energy costly metabolic pathway responsible for increased FPG (Buscemi et al., 2014). Research also found that the gluconeogenic energy consumption in the IR group accounted for approximately one-third of the total resting energy consumption, which is much higher than in the normal weight healthy group (Quaye et al., 2021).

Reduced glycogen uptake and abnormal lipid metabolism were important basic features of IR (Cline et al., 1999; Kelley et al., 2002). According to the Randle cycle theory, using one energy substance leads to the inhibition of other energy substances. The selection of glucose and fatty acids by the body is a dynamic process (Randle et al., 1963), elevated fatty acid levels lead to glucose intolerance, which induces IR, increases lipolytic reactions, reduces mitochondrial fatty acid oxidation in IR populations, leaded to the accumulation of diacylglycerols and ceramides by reverse synthesis, further stimulating protein kinases and inhibiting insulin signaling (Morino et al., 2006; Savage et al., 2007), created a vicious cycle. The IR population had an 80% higher intracellular triglyceride content in myocytes and a 30% lower rate of ATP synthesis in mitochondria than the insulin-sensitive normal population (DiMenna and Arad, 2018). It was further found that under insulin-stimulated conditions (hyperinsulin-orthoglycemic clamp), the rate of mitochondrial synthesis increased by only 5% in the IR population, compared with a 90% increase in the insulin-sensitive normal population (Petersen et al., 2005). In the present study, fat oxidation was significantly higher in the IR group than in the no-IR group during resting conditions and lower in carbohydrate oxidation, suggesting an increased fatty acid supply and inhibited glycogen oxidation in IR.

Despite higher levels of lipid metabolism in the IR group under resting conditions, the IR group had significantly lower lipid metabolic flexibility than the no-IR group during the incremental treadmill test (0.19 ± 0.06 g/min vs. 0.25 ± 0.09 g/min, $p = 0.010$). Metabolic flexibility refers to the ability of the body to respond to changes in metabolic or energy requirements (Kelley et al., 1999). Normal levels of metabolic flexibility play a critical role in maintaining intracellular energy homeostasis (Goodpaster and Sparks, 2017; Smith et al., 2018). Compared to the normal weight populations, the obese IR population have a weaker oxidative regulation of lipid metabolism *in vivo* before and after high-fat diet intervention (Blaak et al., 2006). Impaired flexibility of lipid metabolism also means that FFA cannot be metabolized efficiently, which may lead to enhanced body lipotoxicity and further aggravate IR.

Exercise intensity is usually based on the gold standard of MET: 1 MET is the rate of energy expenditure at resting state, which is approximately equal to 3.5 ml $min^{-1}{\cdot}kg^{-1}$ (Balke, 1960); 1.51–2.99 METs are for low intensity; 3.00–5.99 METs are for moderate intensity; and ≥6 METs are for high intensity (Hibbing et al., 2020). The 1 MET of the obese IR children and adolescents in this study was significantly higher than that of the no-IR group (4.33 ± 0.94 ml $min^{-1}{\cdot}kg^{-1}$ vs. 3.91 ± 0.73 ml $min^{-1}{\cdot}kg^{-1}$), and the resting metabolic equivalent was also higher than the agreed value of 3.5 ml $min^{-1}{\cdot}kg^{-1}$ in both the IR and no-IR groups. Given that METs have strong standardized properties and generalizability (Hibbing et al., 2020), a redefinition is needed when using MET for exercise intensity evaluation in IR or no-IR obese children and adolescents.

In the incremental treadmill test, the MFO of the IR group was significantly smaller than that of the no-IR group, which is consistent with previous studies (Cancino Ramírez et al., 2018); under the same exercise intensity, both the AFO and RFO of the IR group were lower than those of the no-IR group, and the difference increased in significance with the exercise intensity. This phenomenon may be related to the mitochondrial dysfunction in the adipose tissue of the IR group. The IR population exhibited a lower expression of mitochondrial function-related proteins in the subcutaneous adipose tissue than the insulin-sensitive normal population (Xie et al., 2016). The abnormal mitochondrial function in the adipose tissue also inhibits insulin secretion and reduces insulin sensitivity (Luo and Liu, 2016). Hypoxia-inducible factor-1α (HIF1α) is a central regulator of glycolysis during hypoxia (Sato et al., 2019), sirtuin2 (SIRT2) plays an important role in fatty acid oxidation progress (Cha et al., 2017), peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) can regulates lipid metabolism by upregulating the expression of several genes of the tricarboxylic acid cycle and the mitochondrial fatty acid oxidation pathway (Cheng et al., 2018). IR populations have higher expression levels of HIF1α and lower expression levels of SIRT2 than the normal weight populations. Abnormal expression levels of HIF-α and SIRT2 reduce PGC-1α deacetylation, resulting in decreased adipose tissue β-oxidation levels (Krishnan et al., 2012). In obese individuals, the body's lipid metabolism capacity is reduced, the body becomes lipotoxic, and insulin signaling becomes impaired, causing the body to become IR (Morino et al., 2006), which leads to the further accumulation of lipids and subsequently increases obesity.

Exercise is an important prevention method and adjunctive treatment of obesity and chronic diseases (Pedersen and Saltin, 2015). It has a significant effect on improving lipid metabolism disorders, increasing insulin sensitivity, and improving IR in obese children and adolescents (Lee et al., 2012; Marson et al., 2016; Lee et al., 2019). One of the essential ways that exercise improves IR is by increasing lipid oxidation (Pedersen and Saltin, 2006).

Obesity is an important causative factor of IR, and efficient fat loss through exercise was an effective way of improving IR, while FATmax was the optimal fat loss intensity (Jiang et al., 2020). A cross-sectional study found that more fat was burned when exercising at FATmax (45%VO_2max) than at high intensities (70% VO_2max) (Lazzer et al., 2010). The present study also found that the FATmax was significantly lower in the IR group than in the no-IR group (130.9 ± 8.9 vs. 139.9 ± 7.4, $p = 0.040$), which is consistent with previous studies (Suk et al., 2015). This result suggested that lower exercise intensity was required when fat loss exercise interventions were performed in the obese IR population than in the no-IR group. In addition, the HR response during exercise was more significant in the IR group, which could be mainly due to the lower level of cardiorespiratory fitness in the IR group. Cardiorespiratory fitness in children also shows a significant positive correlation with insulin sensitivity, the lower the level of cardiorespiratory fitness, the lower the insulin sensitivity (Slinger et al., 2008; Henderson et al., 2012; Medrano et al., 2020), which also suggested that the lower the insulin sensitivity during exercise, the stronger the cardiovascular response will be.

Different exercise intensities determined the different energy substrates during exercise, and produced different metabolic responses (Fedewa et al., 2014). Low to moderate intensity was the optimal intensity for fat oxidation, while moderate to high intensity was highly significant for carbohydrate consumption (Houmard, 2008). Metabolomic studies have also found that moderate intensity is more beneficial for lipid oxidation than high intensity for the same amount of exercise (Huffman et al., 2014). In the resting state, the energy supply is dominated by lipid oxidation, and the phosphorylase activity in the skeletal muscle increases with the intensity of exercise, leading to an accelerated rate of glycogen breakdown and a gradual increase in the proportion of carbohydrate supply (Romijn et al., 1993). Obesity is an important causative factor of IR in the body, and exercise at FATmax is theoretically the most beneficial for fat elimination (Jiang et al., 2020) and has the most significant effect on IR improvement while controlling other exercise conditions. Tan et al. (Tan et al., 2018) also found that exercise intervention at FATmax for 12 weeks at 3 times per week significantly improves insulin sensitivity.

Limitations

This study mainly used indirect calorimetry to explores energy metabolism characteristics and glycolipid metabolism during incremental load exercise in obese IR and no-IR children and adolescents but did not include normal weight children and adolescents. In addition, protein oxidation was not calculated in this study, despite the low percentage of protein energy supply. The present study initially explored the flexibility of lipid metabolism in the IR group. Although other methods like ^{13}C-based metabolic flux analysis or metabonomics are more advanced, these methods are disadvantage of invasive, extremely labor-intensive, or time-consuming. In addition, in future research, advanced methods can be used to further explore the metabolic flexibility of IR, the mechanisms and potential treatments for exercise to improve IR or type 2 diabetes mellitus.

Conclusion

Significant differences were observed in the resting and exercise states of obese IR and no-IR children and adolescent. The REE of the IR group was significantly higher than in the no-IR group. In constrast, FATmax was lower in the no-IR group, and the HR response during the incremental treadmill test was stronger. The obese IR children and adolescents showed lipid metabolic inflexibility.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Guangzhou Sports Institute (Approval number: 2018LCLL-008) Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CY and ZL conceived the experiments. CY and ZL analyzed the data. CY, ZL, CZ, and XW carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Predicting VO_{2max} in competitive cyclists: Is the FRIEND equation the optimal choice?

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Predicting VO_{2max} in athletes is vital for determining endurance capacity, for performance monitoring, in clinical diagnostic procedures, and for disease management. This study aimed to assess the most suitable equation for predicting VO_{2max} in competitive cyclists. Competitive cyclists (496 males, 84 females, Caucasian, 580 total) were included in the study from 1 January 2014 to 31 December 2019. Only subjects who were actively participating in national or international competitions and who were registered competitive cyclists and part of cycling teams at the time of the measurements were included. Subjects performed an incremental test on a cycle ergometer, and VO_{2max} was measured as indicated by a plateau in VO_2. In addition, four prediction equations (the FRIEND, Storer, Fairbarn, and Jones) were used to estimate VO_{2max}. The predicted VO_{2max} using the FRIEND equation was in good agreement with the measured VO_{2max} in male and female athletes. This was reflected by a high correlation with r = 0.684 for men and r = 0.897 for women (p = 0.000), with ICC = 0.568 (95% CI 0.184, 0.752) for men and ICC = 0.881 (95% CI 0.813, 0.923) for women. Total error was 1.56 and 1.48 ml/min/kg and a minimal bias of−3.6 and −1.1 ml/min/kg (men and women, respectively). Using other equations resulted in a slight decline in agreement with the measured standard. The FRIEND equation predicted VO_{2max} accurately with small total error, small prediction errors, and with the smallest constant error in our study cohort, indicating the potential value of using FRIEND equation also in competitive cyclists. This equation proved to have the highest accuracy both in male and female cyclists.

KEYWORDS

maximal oxygen consumption, performance, FRIEND equation, indirect calorimetry, endurance capacity

Introduction

Cardiopulmonary exercise testing is used to define the functional capacity and prognosis in heart and lung disease patients (Palange et al., 2018). In addition to health measures, it is also used to assess performance abilities in athletes (Balady et al., 2010). Indirect calorimetry in exercise testing is considered the gold standard to detect maximal oxygen uptake (VO_{2max}), but it requires a skilled technician and the use of standardized exercise treadmill protocols or cycle ergometry (Balady et al., 2010). Comparing measured values of VO_{2max} of an athlete to predicted VO_{2max} values of normally active people can lead to misdiagnosis. Since their VO_{2max} is superior, a decline in VO_{2max} could be overlooked if it is compared to non-athletic population prediction values. This could lead to a failure to detect overtraining. Accurately predicted VO_{2max} values are needed to determine whether measured VO_{2max} in an athlete is suboptimal, which can lead to further investigation of the cause.

If indirect calorimetry is not available, different predictive equations are used in evaluating functional capacity in patients and athletes. The generally used equation is the American College of Sports Medicine (ACSM) equation (Glass et al., 2007); however, it is based on VO_{2max} measurements at a submaximal load on relatively small numbers of non-athletic healthy young adults. In addition to some established equations widely used in the past (Jones et al., 1985; Wasserman et al., 1987; Arstila et al., 1990; Storer et al., 1990; Fairbarn et al., 1994), a new FRIEND (The Fitness Registry and the Importance of Exercise National Database) equation was described to have a good prognostic value in heart failure (Kokkinos et al., 2020), coronary artery disease patients (Jang et al., 2020), and in healthy adults (Myers et al., 2017; Kokkinos et al., 2018).

In healthy trained adults, different equations have already been compared to establish the most accurate one for predicting VO_{2max} (Malek et al., 2004), while data in competitive athletes are limited. The most accurate equation for healthy trained adults was the Storer equation (Storer et al., 1990; Malek et al., 2004). Since competitive athletes do not exhibit comparable average VO_{2max} values as trained adults (Balady et al., 2010), this study aimed to assess the most suitable equation for predicting VO_{2max} in competitive cyclists. In addition, we wanted to assess if gender affects the accuracy of the prediction equation used.

Materials and methods

Design

Cardiopulmonary exercise testing with indirect calorimetry was used to determine the VO_{2max} in subjects. We used a cross-validation design as per Malek et al., (2004) to determine which of the equations can estimate the measured VO_{2max} with best precision (Table 1). The cross-validation analyses were based on measuring VO_{2max} and comparing it to predicted VO_{2max} by calculating the constant error (CE, which is the mean difference for actual VO_{2max}-predicted VO_{2max}), Pearson's product–moment correlation (r), standard error of estimate (SEE), and total error (TE) (Table 1). The equations compared to estimate the measured VO_{2max} were selected based on findings using traditionally known equations on aerobically trained men and women (Malek et al., 2004) (Table 2). In addition, the new FRIEND equation was also included in the analysis. Institutional ethical committee approved performing this research.

Subjects

A total of 580 competitive cyclists (496 men and 84 women, all Caucasian) were included in the study. Only subjects who were actively participating in national or international competitions and who were registered competitive cyclists and part of cycling teams at the time of the measurements were included. Data were gathered in five consecutive years (2014–2019) by the same personnel. Informed consent was obtained from all cyclists before starting the procedures.

Procedures

The test subjects had to refrain from intense physical activity 24 hours prior to it. All incremental tests were performed on a cycle ergometer (Cyclus 2, Leipzig, Germany) with their own bike after a 15-min warm-up. Two protocols were used based on age and body mass. They are modified Conconi cycling tests: cyclists under 17 years of age or weighing less than 50 kg started the protocol at 60 Watts and increased 15 Watts every minute (the 60 + 15 W protocol), and cyclists above 17 years of age and weighing more than 50 kg started the protocol at 100 Watts and increased 20 Watts every minute (the 100 + 20 W protocol). The workload was constantly increased until volitional exhaustion, meaning that participants themselves declared when their absolute maximum was reached and the test was to be terminated. The test was also terminated if the cycling cadence dropped below 60. Heart rate (Polar V800, Polar Electro, Kempele, Finland), ventilatory, and gas data (measured with a V2 mask, Hans Rudolph, United States, of appropriate size) were collected during the incremental test with a metabolic cart (K5, Cosmed, Italy). Using breath-by-breath data, the VO_{2max} was determined as the average of the 5-s highest values during the last 30 s of the incremental test. All participants had to reach a plateau in VO_{2max} and RER ˃1.0 for their result to be recognized as maximal exertion and included in this analysis (Howley et al., 1995). The plateau was determined visually by experienced technicians. All measurements were performed in the physiological laboratory, with an ambient temperature of 21°C. The metabolic cart was calibrated prior to each of the measurements.

Statistical analysis

For statistical analysis, SPSS version 25.0 (IBM SPSS Statistics, Chicago, Illinois, United States) was used. Descriptive statistics (average ±standard deviation) were used to represent the data. Four equations were compared: Jones, Fairbarn, Storer, and FRIEND equation. Correlations were calculated using the Pearson correlation coefficient (r). Total error (TE), a measure of a combination of random and systematic error, was calculated. Constant error (CE) was used to determine how much error should be expected if a prediction model was used instead of actual measurement. Finally, standard error of the estimate (SEE), measuring the accuracy of the predictions made by a regression model, was calculated (Supplementary File S1). Dependent *t*-test was used to compare the mean difference between the measured and predicted VO_{2max}. Alpha was adjusted by the Bonferroni procedure. The Bland–Atman test was used for presenting results to evaluate the agreement among measured and predicted VO_{2max} values (Watson and Petrie, 2010; Odor et al., 2017). Intraclass correlation coefficient (ICC) was used to determine the interrater reliability and was calculated using an absolute agreement definition.

Results

The characteristics of the cyclists included in the study are presented in Table 3.

The predicted VO_{2max} varied significantly between the four equations used, and mean values were different from measured mean by + 5.8% to −11,6% in male cyclists and by + 0.5% to −27% in female cyclists. However, we found that the FRIEND equation, when compared with the reference measured VO_{2max}, was the most accurate for predicting VO_{2max} both in men and women.

In men, the predicted VO_{2max} using the FRIEND equation was in the most accurate agreement of all equations compared with the

TABLE 1 Four equations were compared in the cross-validation design: Jones (Jones et al., 1985), Fairbarn (Fairbarn et al., 1994), Storer (Storer et al., 1990), and FRIEND equation (Kokkinos et al., 2018).

Jones	Male	VO_{2max} (l/ min) = (0.046*BH) − (0.021*age) − 4.31
	Female	VO_{2max} (l/ min) = (0.046*BH) − (0.021*age) − 4.93
Fairbarn	Male	VO_{2max} (l/ min) = (0.023*BH) + (0.0117*BW) − (0.031*age) − 0.332
	Female	VO_{2max} (l/ min) = (0.0158*BH) + (0.00899*BW) − (0.027*age) + 0.207
Storer	Male	VO_{2max} (ml/kg)/ min) = (10.51*PO (watt)) + (6.35*BW) + (10.49*age) + 519.3
	Female	VO_{2max} (ml/kg)/ min) = (9.39*PO (watt)) + (7.70*BW) + (5.88*age) + 136.7
Friends	Male	VO_{2max} (ml/kg/ min) = 1.76*(PO (watt)*6.12/kg BW) + 3.5
	Female	VO_{2max} (ml/kg/ min) = 1.65*(PO (watt)*6.12/kg BW) + 3.5

VO_{2max}, maximal oxygen consumption; PO, maximal power output; BW, body weight; BH, body height.

TABLE 2 Prediction models used for cross-validation in this study.

	Analyzed cohort	Protocol	Mean age	Male cyclists		Female cyclists	
				N	Mean VO_{2max}	N	Mean VO_{2max}
FRIEND	Excluded if subjects were diagnosed with (a) a history of cancer (any kind); (b) cardio-vascular disease; (c) chronic obstructive pulmonary disease; (d) chronic kidney disease; and (e) peripheral artery disease. Also excluded were those whose exercise tests were terminated for abnormal clinical findings and/or before achieving voluntary maximal effort (peak respiratory exchange ratio <1.0) and those less than 18 years of age	Not determined	35.9 ± 12.1	3,378	42.43 ± 9.57 mL/min/kg	1,722	23.25 ± 10.01 mL/ min/kg
Storer	Inclusion criteria: sedators, non-smokers, and apparently healthy adults	Start at 0 W + 15 W/min	Ages 20–70, evenly distributed	115	2773.5 ± 603.3 mL/min	114	1612.1 ± 393.8 mL/min
Fairbarn	Exclusion criteria: athletes, use of any medication that could interfere with exercise performance and/or heart rate response (e.g., digoxin, 8-adrenergic blocking drugs, sympathomimetics), abnormal resting ECG, or baseline spirometry findings	Start at 16 or 32 W + 16 W/min	Ages 20–80, evenly distributed	111	51.7 ± 11.4 mL/min/kg for age 20–29; not reported for the whole sample	120	43.9 ± 9.6 mL/min/kg for age 20–29; not reported for the whole sample
Jones	Exclusion criteria: athletes and subjects with history of serious illness or any chronic disorders	Start at 16.3 W + 16.3 W/min	Ages 15–71, evenly distributed	50	Not reported	50	Not reported

measured VO_{2max}, reflected by a correlation with $r = 0.684$ ($p = 0.000$), ICC = 0.881 (95% CI 0.184, 0.752) and total error 1.56 ml/min/kg (Table 4), and a minimal bias of—3.6 ml/min/kg with the limit of agreement −15.52 and 8.32 ml/min/kg (Figure 1), while using other equations resulted in a slight decline in agreement with the measured standard (Table 4).

In women, we observed a wider range in predicted VO_{2max} values than in men (40.05–55.92 ml/min/kg). The predicted VO_{2max} using the FRIEND equation was in very good agreement with the measured VO_{2max}, having a high correlation with $r = 0.897$ ($p = 0.000$), ICC = 0.881 (95% CI 0.813, 0.923) and a total error of 1.48 ml/min/kg (Table 4), and a minimal bias of -1.1 ml/min/kg with the limit of agreement -16.76 and 14.56 ml/min/kg (Figure 1), whereas in men, using other equations resulted in a slight decline in agreement with the measured standard (Table 4).

Discussion

This study evaluated the accuracy of the equations for predicting VO_{2max} in a sample of 496 male and 84 female competitive cyclists. Subjects' characteristics confirmed that cyclists involved in the study were highly trained based on the measured VO_{2max} and relative power output (Faria et al., 2005a; Faria et al., 2005b) (Table 3).

Measuring total error could determine the difference between the measured VO_{2max} (true value) and predicted VO_{2max} (value derived from the equation) in each of the athletes. In the FRIEND equation, the total error was only 1.56 ml/min/kg, whereas in the Jones equation, it was 10.38 ml/min/kg in male cyclists. In the female counterpart, the differences are even greater, up to 31.21 ml/min/kg. VO_2 measurements with an error of >10% are unacceptable (Palange

TABLE 3 Characteristics of cyclists included in the study.

	Males (N = 496)		Females (N = 84)	
Teams	Thirteen competitive cycling teams		Nine competitive cycling teams	
Protocols	Increments of 20 W/min (’50 kg and age ≥17) or 15 W/min (≤50 kg)		Increments of 20 W/min (’50 kg and age ≥17) or 15 W/min (≤50 kg)	
	Mean	Std. Deviation	Mean	Std. Deviation
Age (years)	17.14	2.72	20.18	5.59
Height (cm)	178.67	6.73	166.04	5.57
Body composition				
Body mass (kg)	66.72	7.58	57.45	6.46
Fat mass (%)	9.29	3.87	17.34	4.67
Fat free mass (%)	51.43	1.68	45.98	2.8
Maximal incremental test				
Maximal power output (W)	393.74	56.48	296.05	45.05
Maximal power output (W/kg)	5.90	0.56	5.19	0.79
Maximal heart rate measured (beats/min)	198.46	7.90	194.24	7.85
Maximal HR predicted (beats/min)	202.86	2.72	199.82	5.59
Maximal oxygen consumption (mL/min/kg)	63.43	5.49	54.82	7.02

TABLE 4 Cross-validation of maximal oxygen uptake (VO_{2max}) in male and female competitive cyclists.

Equation	Predicted VO2max (mL/min/kg) (mean)	SD	t	r	SEE (mL/min/kg)	SEE%	TE (mL/min/kg)	TE%	Bland–Altman analysis			Intraclass correlation		
									CE	95% CI		ICC	95% CI	
										Lower	Upper		Lower	Upper
Male cyclists (N = 496)														
FRIEND	67.08	6.08	−17.551	**0.684***	4.013	6%	1.56	2.46%	−3.6	−15.52	8.32	0.568**	0.184	0.752
Storer	73.58	5.78	−50.156	**0.682***	4.023	6.35%	5.53	8.72%	−10.1	−21.43	1.23	0.260**	−0.073	0.595
Fairbarn	60.89	5.12	7.350	−0.049	5.493	8.66%	2.94	4.64%	2.5	−7.54	12.54	−0.044	−0.123	0.037
Jones	53.52	4.08	31.698	−0.036	5.496	8.67%	6.58	10.38%	9.9	1.9	17.9	−0.011	−0.044	0.026
Female cyclists (N = 84)														
FRIEND	55.92	7.99	−2.847	**0.897***	3.126	5.70%	1.48	2.70%	−1.1	−16.76	14.56	0.881**	0.813	0.923
Storer	56.88	7.20	−5.700	**0.892***	3.196	5.83%	1.65	3.01%	−2.1	−16.22	12.02	0.856**	0.666	0.927
Fairbarn	49.30	5.76	6.007	0.142	6.988	12.74%	10.96	20.00%	5.5	−5.79	16.79	0.103	−0.066	0.279
Jones	40.05	5.04	16.325	0.083	7.035	12.83%	31.21	56.95%	14.8	4.92	24.68	0.020	−0.039	0.1

*Correlation is significant at the 0.01 level (2-tailed).
**ICC is significant at the 0.01 level.
*Alpha adjusted by Bonferroni procedure (P 0.05/4 = 0.0125).
(r, Pearson’s correlation coefficient; SEE, standard error of the estimate; TE, total error; CE, constant error; CI, confidence interval; ICC, intraclass correlation coefficient).

et al., 2018), meaning that in male cyclists, the Jones equation is not appropriate. In female cyclists, only FRIEND (2.70%) and Storer (3.01%) equations could be used.

Looking for the strength of association between measured and predicted VO_{2max} in male cyclists, only the FRIEND and Storer equations showed a large positive strength of association to the measured VO_{2max}. In female cyclists, the correlation was even higher than in male counterparts, whereas Jones and Fairbarn equations did not show any significant associations. The predicted VO_{2max} using the FRIEND equation was in very good agreement with the measured VO_{2max}, as supported by the highest ICC in both male and female athletes.

In the present study, the standard error of estimate was used to detect approximately how large the prediction errors (residuals) are for our data set. We found the smallest prediction errors in the FRIEND and Storer equations for both sexes. We can see that the

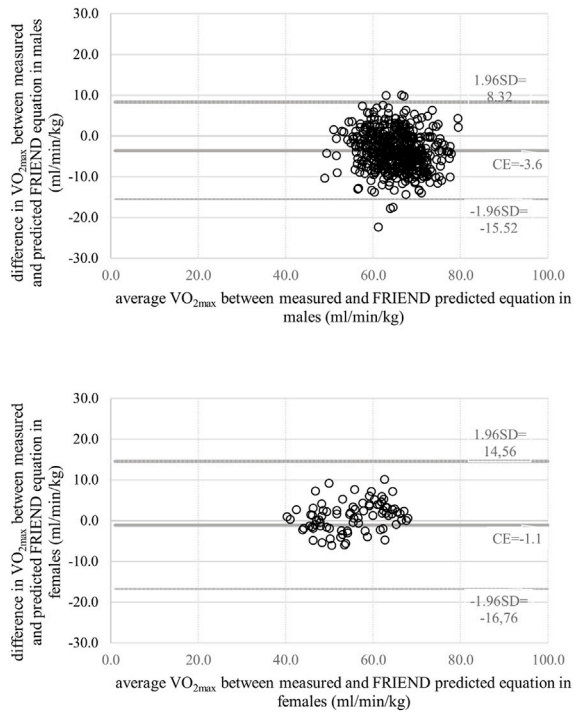

FIGURE 1
Bland–Altman plots comparing measured VO_{2max} and predicted VO_{2max} using the FRIEND equation for men and women.

variability was slightly lower in our female cyclists than in the male cyclists.

In both samples, male and female cyclists, we established that only the FRIEND and the Storer equations show small total error, a significant positive correlation with measured VO_{2max}, and small prediction errors. However, looking at constant error, in the male sample, the Storer equation resulted in large values, meaning that the calculated values deviate consistently from their true value to a much greater extent than in the FRIEND equation. We observed the same finding in female cyclists, but in this sample, the difference was smaller (−1.1 in FRIEND and −2.1 in Storer). In other words, the systematic error was the smallest when using the FRIEND equation for both males and females.

The FRIEND equation clearly seems superior to the Storer equation regarding both sexes, but differences are much smaller in the female sample. This might be due to a smaller sample size, but from the finding of the present study, we can suggest that both equations could be used. A reason why the FRIEND equation has a bigger correlation to measured values in female than in male cyclists could be due to the higher average age of our female samples—there is a smaller difference compared to average age in the FRIEND cohort. Another factor influencing performance is body fat, which can be extremely low in male cyclists and is associated with cycling performance (Jurov et al., 2020). Competitive female cyclists also have less body fat than normally active women (Martin et al., 2001), but their levels are not as extreme as in males, as was also the case in our study. Another advantage of the FRIEND equation is that unlike the Storer equation, age is not part of the calculation; only body weight and power output are.

In the present study, our sample was compared to prediction models (the Storer, Fairbarn, and Jones equations) that proved to be most accurate based on findings by Malek et al., (2004) that compared aerobically trained men and women. In addition, the new FRIEND equation was added to the comparison (Kokkinos et al., 2018). Prediction models made for specific populations may not be appropriate for populations with different characteristics (Zwiren et al., 1991; Kolkhorst and Dolaener, 1994; Malek et al., 2004; Myers et al., 2017; Kokkinos et al., 2018). Our two samples of 496 male and 84 female athletes are homogenous based on age, body composition, fitness, and activity level and are larger than the analyzed cohorts in prediction models (Table 2). The FRIEND registry is based on a larger cohort (3,378 men; mean age 35.9 ± 12.1 years), but considering the youngest age group, which is of interest when elite athletes are involved, the group is of similar size to our study sample (n = 505, age 20–29 years). This cohort is a few times bigger than the ones used in the Jones, Fairbarn, and Storer models, which could be one of the reasons for the better accuracy as shown in this study. Age in the Jones, Fairbarn, and Storer equation is higher than expected in competitive cyclists (Table 2). In the Fairbarn and Jones prediction models, age is also part of the equation. The older the person, the smaller the VO_{2max} (Table 1). We believe this could explain why the Fairbarn and Jones models overestimate VO_{2max}, which results in a positive CE. The FRIEND and Storer models are not based on age, but on power output, which seems more appropriate. They underestimate VO_{2max}, but absolute values of CE are smaller (Table 4; Table A1). In addition, the age span included is quite wide in all three mentioned models. All four models are based on inclusion criteria that used participants who were adults, and the activity level was not specifically determined. However, the Jones and Fairbarn model excluded athletes, and the Storer model included only sedentary individuals (Table 2). Since the FRIEND model did not exclude subjects based on vigorous activity or participation in competition, this might be the reason for the highest accuracy for predicting VO_{2max} in competitive cyclists of the models compared in the present study.

In general, there is a lack of cardiopulmonary testing protocol standardization (Palange et al., 2018), so it is challenging to get large cohorts of subjects with the same protocol, even more so if a specific population is studied, like competitive cyclists. The FRIEND registry is based on data obtained from different laboratories, and a specific protocol was not defined as part of the inclusion process. This is a disadvantage of the FRIEND equation, as the type of protocol can influence the VO_{2max} value (Midgley et al., 2008). We believe that using the same protocol as in our sample could result in even better accuracy of the predicted equation. Regardless of the lack of protocol standardization in the FRIEND prediction model, the equation proved to be the most accurate in our study. We assume that the large sample size in the FRIEND model could be the most important factor that affects the accuracy of the FRIEND equation.

Limitations

There are some limitations that should be considered. Although the male sample in this study is the biggest sample of male competitive cyclists used in common studies, the female sample size is smaller. There are fewer female competitive cyclists in general, and to gather more data, we believe different laboratories should combine their data. Still, to the best of our knowledge, this is the biggest sample of female competitive cyclists using the same protocol, measurement equipment, and data collection procedures. In addition, the mean age of the male cyclists included in this study was slightly under 18 years, and we used prediction equations based on adults.

Conclusion

Accurate prediction of VO_{2max} is vital in sports medicine (Sartor et al., 2013). For practitioners with no access to indirect calorimetry, it is the only way of assessing oxygen uptake at maximal exercise tolerance (Palange et al., 2018). Equations for VO_{2max} are useful also in field testing, which are very common in sports medicine and where indirect calorimetry is not always possible. When VO_{2max} measurement is available, predicted VO_{2max} can help identify possible decline in maximal values due to health impairment, like in cases of heart and lung diseases (Frederix, 2014). Since competitive athletes have a higher VO_{2max} than normally active adults, inaccurate predicted values can lead a physician to underestimate the severity of measured VO_{2max} or fail to recognize it at all. We demonstrated that the FRIEND equation predicted VO_{2max} most accurately with small total error, small prediction errors, and with the smallest constant error in our study cohort, indicating the potential value of using the FRIEND equation also in competitive cyclists. This equation proved to have the highest accuracy both in male and female cyclists. Since endurance athletes (like cyclists, triathletes, long distance runners) have similar body composition and endurance capacity requirements (Millet et al., 2009; Santos et al., 2014), this model might be appropriate also in the wider group of athletes. Further research is required to support or challenge our findings, to determine whether this model can be utilized in endurance disciplines, and to establish if athletes of other modalities (power disciplines and esthetic sports) show any dissimilarities.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization, IJ and MC; methodology, IJ; formal analysis, IJ; investigation, IJ; data curation, IJ; writing—original draft preparation, IJ; writing—review and editing, IJ, JT, and MC; supervision, JT. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Cardiorespiratory optimal point as a submaximal evaluation tool in endurance athletes: An exploratory study

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Introduction: The cardiorespiratory optimal point (COP) represents the lowest minute ventilation to oxygen consumption ratio (VE/VO2) and can be estimated during a CPET at submaximal intensity when an exercise test until volitional fatigue is not always advisable (i.e., a conflict zone where you cannot be confident of the security because near-competition, off-season, among other). COP's physiological components have not been wholly described yet. Therefore, this study seeks to identify the determinants of COP in highly trained athletes and its influence on maximum and sub-maximum variables during CPET through principal c omponent analysis (PCA) (explains the dataset's variance).

Methods: Female (n = 9; age, 17.4 ± 3.1 y; maximal VO2 [VO2max]), 46.2 ± 5.9 mL/kg/min) and male (n = 24; age, 19.7 ± 4.0 y; VO2max, 56.1 ± 7.6 mL/kg/min) athletes performed a CPET to determine the COP, ventilatory threshold 1 (VT1) and 2 (VT2), and VO2max. The PCA was used to determine the relationship between variables and COP, explaining their variance.

Results: Our data revealed that females and males displayed different COP values. Indeed, males showed a significant diminished COP compared to the female group (22.6 ± 2.9 vs. 27.2 ±3.4 VE/VO2, respectively); nevertheless, COP was allocated before VT1 in both groups.

Discussion: PC analysis revealed that the COP variance was mainly explained (75.6%) by PC1 (expired CO_2 at VO2max) and PC2 (VE at VT2), possibly influencing cardiorespiratory efficiency at VO2max and VT2. Our data suggest that COP could be used as a submaximal index to monitor and assess cardiorespiratory system efficiency in endurance athletes. The COP could be particularly useful during the offseason and competitive periods and the return to the sports continuum.

KEYWORDS

musculoskeletal and neural physiological phenomena, exercise test, oxygen consumption, anaerobic threshold, athletic performance, physical endurance

Introduction

It has been proposed that the more important physiological determinants of exercise performance in all-out performances lasting 21–60 min are maximal oxygen consumption (VO2max), lactate threshold, and movement economy (Midgley et al., 2007). Physiological determinants are routinely assessed through a maximal cardiopulmonary exercise test (CPET); however, a CPET may be problematic under specific athletic scenarios, such as after a period of training cessation or injury. Of note, 66%–91% of athletes return to sports activities after a lower extremity surgery, while a return to pre-injury level performance is possible in 31%–68% of all cases (Vereijken et al., 2020). The strategic assessment of risk and risk tolerance proposes a three-step model to help return to sports decision-making (Shrier, 2015). Particularly, step 2 of the functional test estimates the stress and how much stress can tolerate the injured tissue (Shrier, 2015). However, this strategy was designed to evaluate the functional capacity of the joints and bones. In contrast, cardiorespiratory capacity evaluation throughout a maximal effort test could be problematic in injured athletes during the early stages through the return to sport continuum (Shrier, 2015; Ardern et al., 2016).

Besides, considering injuries (Shrier, 2015) and detraining periods (Mujika and Padilla, 2000a; Mujika and Padilla, 2000b), it could be helpful to evaluate exercise performance during these training cessations. Classically, endurance performance is evaluated through a CPET, and ventilatory thresholds (VTs) have been used to delineate moderate to heavy exercise intensity domains; non-etheless, their reliability is questionable due to the multiple methods used to obtain those calculations (Jamnick et al., 2020). Exercise intensity domains have been useful in manipulating training intensity in athletes who perform close to 80% of their training at low-intensity (BILLAT et al., 2003). Sandbakk et al. (2011) reported that world-class skiers performed 30% more low and moderate-intensity training than national-class skiers. Therefore, reliable submaximal measurements or tests could benefit athletes, providing helpful information to normalize exercise intensity (Sandbakk et al., 2011), which is needed for physiological adaptations to achieve improved performance (Midgley et al., 2007). Hence, it is necessary to track performance-related variables or even to estimate the cardiorespiratory maximum values through submaximal tests, which could help to know how the cardiorespiratory system of endurance athletes could be affected during the off-season and competitive periods, but without the need to perform the maximal effort.

The cardiorespiratory optimal point (COP) is an index obtained/calculated from a submaximal CPET, capable of evaluating the interaction between cardiovascular and respiratory systems through VO2 efficiency (Ramos et al., 2012). COP has been defined as the lowest minute ventilation VE/VO2 ratio in a given minute during a CPET (Ramos et al., 2012). Commonly, the COP is located before the first ventilatory threshold (VT1) (Ramos et al., 2012; Ramos and Araújo, 2017; Silva et al., 2018) and has been interpreted as the lowest VE required to extract 1 L of oxygen, which showed the efficiency of the breathing response to consume oxygen during exercise (Ramos et al., 2012; Ramos and Araújo, 2017; Silva et al., 2018). Importantly, it has been shown that COP could predict the mortality risk independent of sex and comorbidities (Ramos and Araújo, 2017), and to assess the exercise performance of professional soccer players (Silva et al., 2018). Of note, preliminary evidence suggests no correlation of COP with ventilatory thresholds (VTs) or VO2max (Silva et al., 2018; Charitonidis et al., 2020). Nevertheless, the reported absence of correlation might not be able to explain a possible influence and COP variance on multiple combinations of cardiovascular, respiratory, and metabolic variables. In addition, the COP, due to its location at low-intensity (Ramos et al., 2012; Ramos and Araújo, 2017; Silva et al., 2018), could be a helpful tool for estimating and monitoring changes in metabolic and cardiorespiratory variables associated with high-performance variables at a low time-effort ratio in athletes who perform >80% of their training at low-intensity (Seiler, 2010; Stöggl and Sperlich, 2015). Principal Component Analysis (PCA), a statistical method applied to data to reduce the number of variables in principal components, could explain the most variance of specific data helping to determine critical variables (Ringnér, 2008). Thus, considering that there is no compelling evidence on the determinants of COP and how it affects cardiorespiratory performance variables; the present study aimed to seek the determinants of COP in highly trained athletes and its influence on maximum and sub-maximum variables during CPET through PCA.

Methods

Experimental approach to the problem

We performed a study to assess the COP in highly trained athletes (McKay et al., 2022) during an incremental CPET trial and its influence on maximum and sub-maximum variables through PCA. The CPET was performed on a cycle ergometer. In addition, resting metabolic rate and pulmonary function were determined to gather control variables required for enrollment. The PCA was performed with 72 continuous numerical variables obtained during CPET.

Participants

Thirty-three highly trained endurance athletes (female, n = 9; male, n = 24) voluntarily participated in this study. All-female participants were measured in the same stage of the menstrual cycle in different weeks. Exercise testing sessions were conducted between 08:00 and 17:00 h. Previously, all participants and parents of underage athletes were carefully informed about the experiment procedures and the possible risks associated with their participation in the study. They were instructed to refrain from consuming drugs, ergogenic aids, foods, or substances that alter autonomic control or sports performance 48 h before the maximum exercise test. A signed informed consent or assent document was obtained from parents and/or legal guardians, a document that attests to informed consent from a parent and/or legal guardian for study participation that is in accordance with the latest version of the Declaration of Helsinki. All protocols were evaluated by the ethical committee of Universidad Mayor (#169_2019).

Experimental procedure

All participants had a background in endurance activities (medium and long-distance swimmers, road bikers, and long-distance runners)

and were part of the regional team (train >3 h per day, 6 days per week, and minimum 5 years of training with a background in national competitions, being classified in Tier three or "Highly Trained/National Level" according to the Participant Classification Framework (McKay et al., 2022). Exclusion criteria considered for enrollment were: (i) potential medical problems or a history of ankle, knee, or back injury; (ii) any lower extremity reconstructive surgery in the past 2 years or unresolved musculoskeletal disorders; (iii) history of chronic obstructive or restrictive pulmonary diseases and/or altered spirometry on the day of the pre-exercise session (forced expiratory volume at first second (FEV1)/vital capacity (VC) <70, FEV1<80% of predicted value or VC < 80% of predicted value). Inclusion criteria were: i) Being part of the regional team with a background in endurance performance, ii) absence of cardiopulmonary or electrocardiogram (ECG) alterations related to the disease or autonomic dysfunction (as an indicator of overtraining [data not showed]). Participants were familiarized with the test procedures before the measurements were taken. All participants were subject to the same warm-up muscle actions before the exercises (Andrade et al., 2015). The coaches were asked to give the athletes 24 h of rest, and the day before each experimental condition, participants were instructed to (i) have a good night's sleep (~8 h) and (ii) use the same athletic shoes and clothing during the protocols.

Prior to CPET, height, body mass, ECG, and clinical spirometry (VC; peak expiratory flow [PEF]; peak inspiratory flow [PIF]; FEV1; FEV1/VC; forced expiratory flow at 25% [FEF 25], 50% [FEF 50] and 75% [FEF 75] of VC) were taken. Height was measured using a wall-mounted stadiometer (HR-200, Tanita, Japan) to the nearest 0.1 cm. Body mass was measured to the nearest 0.1 kg using a digital scale (BF-350, Tanita, IL, United States). BMI was calculated as body mass/height2.

Cardiopulmonary exercise test (CPET) and cardiorespiratory optimal point

Exercise testing was supervised by an experimented technician according to the American Thoracic Society Guidelines (Weisman et al., 2003). All participants performed the CPET according to the modified Astrand ramp protocol using Convival CPET cycle ergometer (Lode, Netherlands). The cycle ergometer seat was set for each participant (e.g., seat and bar height), prior to each testing session. CPET was performed to determine COP, VO2max, and ventilatory threshold 1 (VT1) and 2 (VT2) and was similar to what has been previously described (Beaver et al., 1986). Briefly, before the maximal test, the participants had a rest time of 5 min on the cycle ergometer, then performed 5 min of warm-up and at an intensity of 25 W. The test started at 50 W, and the workload was increased by 25 W/min until they could not maintain the prescribed cycling frequency of 70 rpm for more than five consecutive seconds (Fletcher et al., 2013). During the test, participants breathed through a valve (Hans Rudolph, United States), and for expired and inspired gas collection and analysis, the Quark CPET metabolic cart (COSMED, Italy) was used. The COP was defined as the lowest oxygen ventilatory equivalent value (VE/VO2 ratio), obtained from an average of six 10-s windowing samples in a given minute, similar to what has been previously described (Ramos et al., 2012). Before each trial, the system was calibrated with a mixture of O2 and CO_2 known (O2 15%, CO_2 5%, N2 balanced; Carburos Metálicos, Barcelona, Spain). Flowmeter calibration was performed using a certified 3 L calibration syringe. VT1 was in the VCO_2 vs. VO2 panel when the intersection between the two linear segments occurs (Beaver et al., 1986) when VE/VO2 begins to increase after being constant or slightly decreasing while VE/VCO_2 has been flat or slightly decreasing (Wasserman, 1984). VT2 was in VE vs. VCO_2 panel when the intersection between the two linear segments occurred (Beaver et al., 1986) with an increase in both VE/VO2 and VE/VCO_2 (Wasserman, 1984).

Pulmonary function

Pulmonary functions were assessed according to both the American Thoracic Society and the European Respiratory Society consensus and similar to what has been previously described. Briefly, all participants were asked to exert maximum effort during forced breathing. Results were derived from three repeated measurements, with between-maneuver variation <5% or 200 mL in forced vital capacity (FVC) and forced expiratory volume at first second (FEV1). The maximal mid-expiratory flow was selected from the best maneuver, that is, the maneuver with the largest sum of FVC and FEV1. We used the maximal expiratory curve to calculate FVC, PEF, PIF, FEV1, FEV1/VC, FEF 25, FEF 50, and FEF 75 of VC. All recordings were performed using Quark PFP spirometer (COSMED, Italy).

Resting metabolic rate

The resting metabolic rate (RMR) was performed by indirect calorimetry using Quark CPET metabolic cart (COSMED, Italy); accordingly, to has been previously described. Briefly, the participants were instrumentalized with an oronasal mask (7450 Series Silicone V2, Hans Rudolph, Kansas City, United States) for expired and inspired gas collection and analysis (Quark CPET metabolic cart; COSMED, Roma, Italy). Nevertheless, before the measurement, the participant took a rest of 30 min in supine positions. After, the recording started, the total time was 40 min, where the first 5-min were discarded as part of the acclimatization period, and the calculation of respiratory quotient (RQ), protein oxidation, carbohydrates, and lipids were calculated from the remaining 35 min. Protein oxidation, carbohydrates, and lipids were expressed as kcal/day and as % of the total resting metabolic rate. The RMR measurement was performed in a specially conditioned room isolated from noise at a temperature of 23°C and 50% of humidity. The RMR was evaluated between 8:00 to 10:00 a.m. For every three measurements, the metabolic cart was re-calibrated with a known calibration gas (O2 15%, CO_2 5%, N2 balanced). The recording and analysis were performed with OMNIA, Cardiopulmonary Diagnostic Suite v 1.4 (Quark CPET metabolic cart; COSMED, Roma, Italy).

Statistical analysis

Data are presented as mean ± standard deviation. Normality (Shapiro-Wilk test) and homoscedasticity (Levene test) tests were performed. To compare both groups, unpaired t-test or Mann-

TABLE 1 Demographics, cardiopulmonary, resting metabolic rate, and pulmonary function.

		Female (n = 9)	Male (n = 24)
Characteristics			
	Age (years)	17.44 ± 3.13	19.71 ± 4.01
	Height (cm)	160 ± 5.59****	175.4 ± 9.04
	Weight (Kg)	56.62 ± 7.14**	69.92 ± 12.88
	BMI	22.12 ± 2.69	22.56 ± 2.67
Cardiopulmonary			
	VO2max (ml·kg-1·min-1)	46.17 ± 5.87**	56.05 ± 7.57
	VO2pred (%)	125.9 ± 14.53	133.2 ± 20.03
	RER	1.161 ± 0.12	1.185 ± 0.08
	VT1 (ml·kg-1·min-1)	30.03 ± 4.85**	38.17 ± 7.33
	VT1%VO2max	65.33 ± 8.99	68 ± 9.18
	VT2 (ml·kg-1·min-1)	37.43 ± 5.81**	47.2 ± 8.08
	VT2%VO2max	81.44 ± 8.97	84.25 ± 9.84
Resting metabolic rate			
	RMR (Kcal/day)	1834 ± 390**	2348 ± 362.4
	FAT%	58.94 ± 20.49	55.35 ± 25.06
	CHO%	41.06 ± 20.49	44.65 ± 25.06
Spirometry			
	FVC (L)	3.803 ± 0.55****	5.444 ± 1.04
	FVCpred (%)	108.6 ± 10.08	105.9 ± 9.73
	FEV1 (L)	3.299 ± 0.5****	4.515 ± 0.72
	FEV1pred (%)	105.8 ± 10.92	102.4 ± 10.06
	PEF (L/s)	6.517 ± 1.02***	8.684 ± 1.4

Values are shown as mean ± standard deviation. BMI: body mass index; VO2max: maximal oxygen consumption; VO2pred: Predicted maximal oxygen consumption; RER: respiratory exchange ratio; VT1: ventilatory threshold one; VT1%VO2max: distance from VT1 to VO2max in percentage; VT2: ventilatory threshold 2; VT2%VO2max: distance from VT2 to VO2max in percentage; RMR: resting metabolic rate; FAT%: percentage of energy from fat; CHO%: percentage of energy from carbohydrate; FVC: forced vital capacity; FVCpred: predicted forced vital capacity; FEV1: forced expiratory volume; FEV1pred: predicted forced expiratory volume in 1 s; PEF: peak expiratory flow. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and ****$p < 0.0001$.

Whitney tests were performed accordingly to data distribution. The α level for all statistics was set as $p < 0.05$. All statistical calculations were performed by GraphPad Prism 9.0 (GraphPad software Inc, CA, United States).

Multivariable correlations and principal component analysis (PCA)

To determine the contribution of different maximal and submaximal variables to explain the COP, we used a PCA to define groups of variables, which could explain the COP variance (Di Carlo et al., 2015). Data from all subjects were organized into an "n" x "m" matrix (with no missing entries), with "n" rows indicating observations (subjects) and "m" columns representing cardiorespiratory, metabolic, and morphological variables (dimensions), generating a 72 × 72 matrix. A Heatmap of the correlation matrix was generated from computed Pearson-r values for every pair of datasets using GraphPad Prism software v9 (GraphPad software Inc, CA, United States). Only numerical continuous variables are considered for multivariable analysis and dimensional reduction, and detailed information of Pearson-r values and the variables used for analysis are depicted in Supplementary Table S1.

PCA was performed after data standardization of the 72 × 72 matrix and eigenvalue decomposition. Data standardization was performed by computing z-scores according to the formula:

$$zi = \frac{(\mathrm{xi} - \mu)}{\sigma}$$

where zi corresponds to the z-score of every individual value, xi to raw individual values, and μ and σ to the mean and standard deviation of datasets, respectively (Jolliffe et al., 2016). Data standardization, eigenvalues, component loadings, and PC scores were calculated using GraphPad Prism software v9 (GraphPad software Inc, CA, United States). For PCA, only the eigenvalues higher than one were considered as significant, according to the Kaiser's rule (Kaufman and Dunlap, 2000). The first component (PC1) accounts

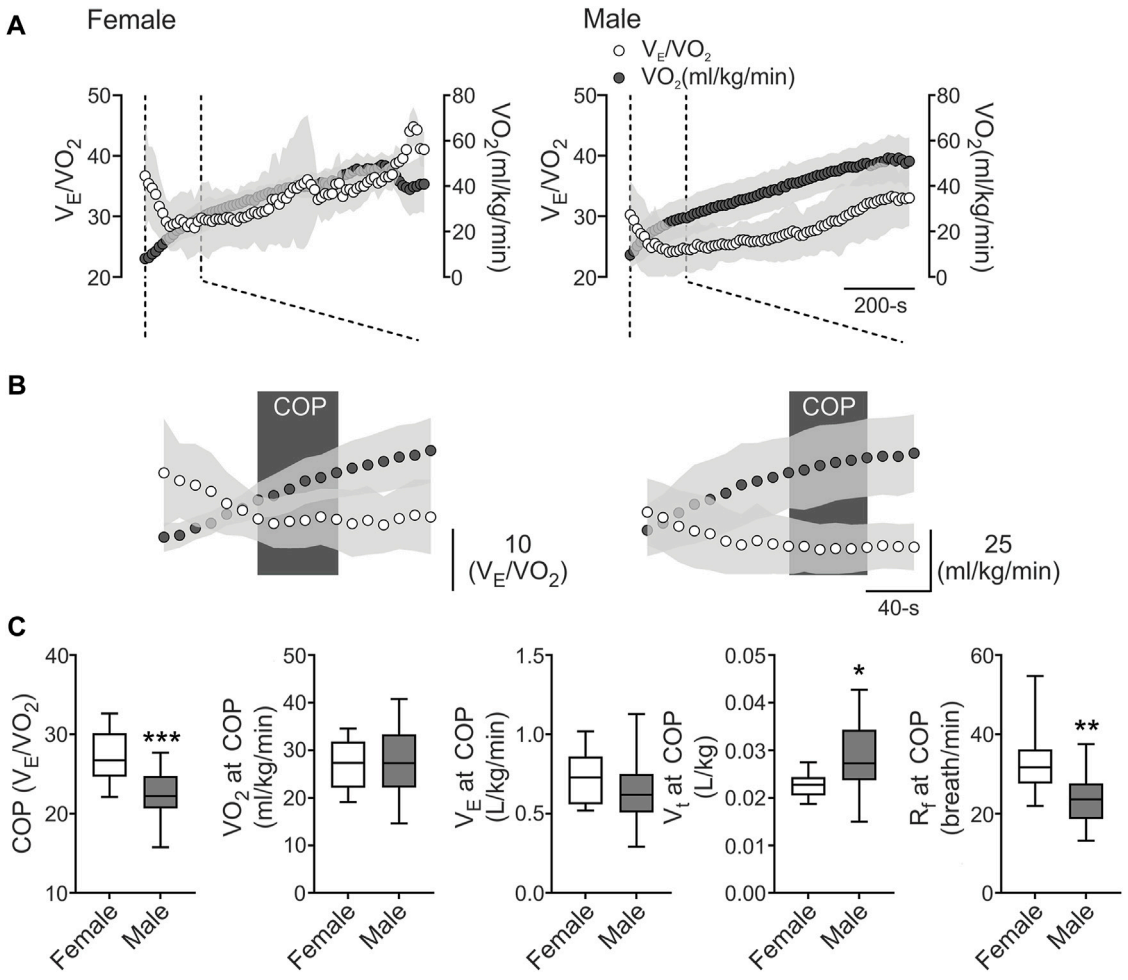

FIGURE 1
The cardiorespiratory optimal point between females and males. **(A)**, Representative VE/VO2 and VO2 recording from one female and one male well-trained athlete. **(B)**, Representation of cardiorespiratory optimal point (COP) determined by minute ventilation/oxygen consumption (VE/VO2). Note that males displayed a lower COP value compared to female participants. **(C)**, Summary data of COP and VO2, VE, tidal volume (VT), respiratory frequency (Rf) at COP (from left to right, respectively). Male participants displayed a higher COP and VO2 at COP and VT compared to female athletes. Also, males displayed a decrease in Rf compared to females. Unpaired T-test, *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. Female, $n = 9$; and Male, $n = 24$.

for most of the total variance of data, and it is associated with the largest eigenvalue; PC2 accounts for as much as possible of the remaining variance, and so on (Di Carlo et al., 2015). Component loadings are equal to a Pearson correlation between the principal component, and a quantified variable is a set of optimal weights (Supplementary Table S2). PC scores were plotted in a biplot to display the information of all individuals as points in the same space as the variables (Di Carlo et al., 2015). After PCA, biplots were created to illustrate the relationship between the variables in the space of selected components.

Results

Baseline physiological variables

Baseline physiological data of demographic, cardiopulmonary, metabolic, and pulmonary function are shown in Table 1. All athletes reached VO2max over their predicted values, and pulmonary function showed normal volumes and capacities of the lungs (Table 1). Our data revealed that COP was located before VT1 (Figures 1A, B), and the COP value in male and female athletes was between 22 and 30 VE/VO2 ratio (22.64 ± 2.95 vs. 27.24 ± 3.36 VE/VO2, respectively) (Figures 1B, C). The cardiorespiratory and metabolic data at COP are shown in Table 2. After VTs and VO2max identification, we assessed cardiorespiratory and metabolic variables during these stages, which are depicted in Table 3.

Multiple regression analysis and PCA

To study the possible relationship of 72 cardiorespiratory, metabolic, and anthropometric variables during VT2 and VO2max (related performance variables) with the COP among the sex of individuals, we computed Pearson R-values for every pair of values in a multivariable correlation matrix (Figure 2A). Our analysis revealed that COP was related, positive and negative, with several cardiorespiratory variables (Figures 2A, B).

TABLE 2 Cardiopulmonary exercise test variables at COP.

	Female (n = 9)	Male (n = 24)
RfCOP	23.36 ± 5.90	33.27 ± 9.16***
VTCOP	1.914 ± 0.49	1.300 ± 0.28**
IVCOP (L)	1237 ± 257.7	1836 ± 494.2 **
VO2COP	1,911 ± 451.7	1,534 ± 326.7*
RERCOP	0.8386 ± 0.05	0.8148 ± 0.09
VE/CO2COP	32.61 ± 4.6	27.86 ± 2.92 **
HRCOP (beats·min-1)	135.5 ± 9.47	120.8 ± 15.88 **
VO2/HRCOP (ml·beats-1)	11.27 ± 2.1	15.82 ± 3.22 ***
PetO2 (mmHg)	96.92 ± 4.66	89.93 ± 5.76 **
PetCO2 (mmHg)	37.47 ± 4.98	42.01 ± 4.09 **
FAT%COP	54.96 ± 15.76	62.78 ± 28.27
CHO%COP	45.04 ± 15.76	37.22 ± 28.27
TiCOP (s)	0.8541 ± 0.158	1.284 ± 0.37 ****
TeCOP (s)	1.086 ± 0.33	1.498 ± 0.45 **
TtotCOP (s)	1.939 ± 0.47	2.782 ± 0.81 **
Ti/TtotCOP	0.4466 ± 0.03	0.4621 ± 0.02
VD/VTCOP	0.1953 ± 0.02	0.184 ± 0.02
VT/TiCOP	1.536 ± 0.22	1.566 ± 0.43

Values are shown as mean ± standard deviation. RfCOP: respiratory frequency at COP; VTCOP: tidal volume at COP; VECOP: ventilation at COP; IVCOP: inspiratory volume at COP; VO2COP: absolute oxygen consumption at COP; RERCOP: respiratory exchange ratio at COP; VE/CO2COP: ventilatory equivalent for carbon dioxide at COP; HRCOP: heart rate at COP; VO2/HRCOP: oxygen pulse at COP; PetO2: end tidal partial pressure of oxygen at COP; PetCO2: end tidal partial pressure of carbon dioxide at COP; FAT%COP: percentage of energy from fat at COP; CHO%COP: percentage of energy from carbohydrate at COP; TiCOP: inspiratory time at COP; TeCOP: expiratory time at COP; TtotCOP: respiratory cycle duration at COP; Ti/TtotCOP: inspiratory time divided by respiratory cycle duration at COP; VD/VTCOP: dead space at COP; VT/TiCOP: ventilatory drive at COP. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and ****$p < 0.0001$.

PCA revealed 12 principal components; however, only PC1 to PC6 were considered as significant (eigenvalues higher than 1, Figures 2C, D) and explained 94.4% of the total variance of data (Figure 2C). Nevertheless, PC1 contributed 47.1%, and PC2 to 28.5% of the total variance (Figure 2C). Loadings (or the contribution of variable "m" to PCn) of each variable to PC1 and PC2 is depicted in Figure 2D and we also generated biplots showing the loadings of each variable to the six first PCs (Figure 2E), based on Pearson-r values of between variables at PC1 and PC2 (Supplementary Table S3), revealing that CO_2exp at VO2max majorly contributed to PC1; and VE at VT2, to PC2 (Figure 2B). We plotted PC scores in a biplot, depicting all individuals distinguished by sex (Figure 2F).

Discussion

The purpose of the study was to resolve the determinants of COP in highly trained athletes and its influence on maximum and sub-maximum variables during CPET through PCA. The main findings of this study were: i) the COP was located before VT1; ii) PCA reveals that COP could influence CO_2exp at VO2max (PC1) and VE at VT2 (PC2). Therefore, these results strongly suggest that COP influences cardiorespiratory performance-related variables at VT2 and VO2max. Further, it is possible that during long-term cessation training, due to, i.e., injury, the COP could be considered used during non-maximal CPET to estimate how much the athlete has been affected by the stop of training, suggesting that the COP could be used during rehabilitation periods.

COP characteristics

The VE/VO2 minimum value (i.e., COP) could be considered a submaximal calculation for the best integration between the circulatory and pulmonary systems (Ramos et al., 2012). Ramos and Araujo (2017) conducted a study that included 3,331 subjects with and without chronic diseases, where they showed three COP categories, defined by the cut-off values < 22, 22-30, and >30 VE/VO2 (Ramos and Araújo, 2017). Importantly, when COP is > 30, it is a good predictor of all-cause mortality independently or in combination with lower VO2max, compared to those with <22 value (Ramos and Araújo, 2017). Our data revealed that COP was located before VT1 and VT2, consistent with previous reports in the non-athlete population (Ramos et al., 2012) and in professional soccer players (Silva et al., 2018). Importantly, our results depicted that both female and male athletes showed values between 22 and 30 VE/VO2 ratio, which was a cutoff classified as moderate, evidencing a good interaction between circulatory and respiratory function (Ramos and Araújo, 2017). However, although this sex-related difference is accordingly to previous reports, its dissimilarity only has been shown

TABLE 3 Sex-related cardiopulmonary differences.

	VT1						VT2						VO2max					
Variables	Female			Male			Female			Male			Female			Male		
t%	44.67	±	9.1	50.25	±	16.08	68.22	±	12.23	74.92	±	13.42	---		---	----		---
Rf	36.09	±	9.57	29.69	±	7.09*	41.89	±	8.72	36.33	±	7.86	52.46	±	9.86	51.18	±	7.63
VT	1.367	±	0.25	2.266	±	0.5****	1.572	±	0.17	2.56	±	0.52****	1.86	±	0.23	2.8	±	0.52****
VE	47.07	±	7.63	64.37	±	10.57****	64.76	±	10.52	90	±	16.08***	96.47	±	19.58	140.6	±	23****
IV	1318	±	226.6	2256	±	454.2****	1538	±	164.7	2575	±	486.2****	1863	±	249.2	2860	±	517.6****
VO2	1687	±	269	2624	±	477****	2106	±	278.3	3251	±	539.7****	2599	±	373.6	3863	±	520.4****
VCO_2	1468	±	212.6	2421	±	487.2****	2052	±	308.4	3322	±	692.9****	2885	±	369.7	4406	±	660.9****
RER	0.8721	±	0.05	0.92	±	0.056*	0.9752	±	0.08	1.017	±	0.07	1.12	±	0.08	1.14	±	0.07
VE/VO2	28.1	±	3.58	24.74	±	2.4**	31.06	±	5.3	27.79	±	2.89*	37.66	±	9.14	36.41	±	3.54
VE/VCO_2	32.33	±	4.66	27.02	±	3.31***	31.9	±	5.03	27.42	±	3.15**	33.65	±	6.63	32.02	±	3.46
METs	8.58	±	1.39	10.91	±	2.09**	10.72	±	1.62	13.49	±	2.31**	13.19	±	1.68	16.01	±	2.17**
HR	145.9	±	13.1	146.4	±	13.93	170	±	9.46	169.7	±	15.33	186.9	±	5.47	189.5	±	9.62
VO2/HR	11.58	±	1.74	17.99	±	3.17****	12.44	±	1.9	19.24	±	3.07****	13.94	±	2.15	20.45	±	2.92****
PetO2	97.73	±	4.8	93.34	±	4.2*	100.6	±	4.94	96.95	±	4.13*	105.2	±	4.92	104.4	±	3.17
PetCO2	37.86	±	4.9	43.56	±	4.61**	38.39	±	5.21	43.39	±	4.27**	37.21	±	5.48	38.83	±	3.65
FAT	5202	±	2387	4953	±	3235	2468	±	2647	1576	±	2456	114	±	245.3	135.9	±	630.3
CHO	6598	±	1856	13617	±	4628****	12613	±	3000	21944	±	5149****	19054	±	2433	28536	±	3759****
FAT%	43.63	±	16.21	27.84	±	18.28*	16.15	±	16.94	7.265	±	11.12		±	1.03		±	1.6

(Continued on following page)

10.3389/fphys.2023.1087829

TABLE 3 (*Continued*) Sex-related cardiopulmonary differences.

Variables	VT1						VT2						VO2max					
	Female			Male			Female			Male			Female			Male		
												0.5			0.3512			
CHO%	56.37	±	16.21	72.16	±	18.28*	83.85	±	16.94	92.74	±	11.12	99.5	±	1.03	99.65	±	1.6
Ti	0.8054	±	0.17	1.015	±	0.23*	0.6928	±	0.11	0.85	±	0.2*	0.57	±	0.09	0.59	±	0.1
Te	0.9854	±	0.33	1.124	±	0.26	0.8035	±	0.2	0.9	±	0.22	0.62	±	0.14	0.63	±	0.1
Ttot	1.791	±	0.48	2.139	±	0.47	1.496	±	0.3	1.74	±	0.41	1.19	±	0.22	1.21	±	0.19
Ti/Ttot	0.4565	±	0.03	0.4747	±	0.02	0.4675	±	0.03	0.49	±	0.03	0.48	±	0.03	0.49	±	0.02
VD/VT	0.1981	±	0.02	0.1906	±	0.02	0.2037	±	0.03	0.2	±	0.02	0.22	±	0.03	0.24	±	0.02
VT/Ti	1.713	±	0.18	2.265	±	0.38***	2.31	±	0.32	3.1	±	0.57***	3.35	±	0.67	4.85	±	0.82****
HRR	56.63	±	14.08	53.65	±	13.07	32.6	±	9.94	30.35	±	14.56	15.65	±	6.26	10.57	±	10.1
SV	92.36	±	14.83	140.4	±	24.18****	87.3	±	14.5	132.8	±	22.3****	85.81	±	13.32	126.4	±	18.53****
Power	155.56	±	39.09	151.04	±	46.90	250.00	±	39.53	291.46	±	34.12*	308.33	±	35.36	342.08	±	30.99**

Values are shown as mean ± standard deviation. t%: difference from VO2max in percentage; Rf: respiratory frequency; VT: tidal volume; VE: ventilation; IV: inspiratory volume; VO2: absolute oxygen consumption; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; VE/O2: ventilatory equivalent for oxygen; VE/CO_2: ventilatory equivalent for carbon dioxide; VO2/kg: relative oxygen consumption; METs: metabolic equivalent of task; HR: heart rate; VO2/HR: oxygen pulse; PetO2: end tidal partial pressure of oxygen; PetCO2: end tidal partial pressure of carbon dioxide; FAT%: percentage of energy from fat; CHO%: percentage of energy from carbohydrate; Ti: inspiratory time; Te: expiratory time; Ttot: respiratory cycle duration; Ti/Ttot: inspiratory time divided by respiratory cycle duration; VD/VT: dead space; VT/Ti: ventilatory drive; HRR: heart rate reserve; SV: stroke volume. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and ****$p < 0.0001$.

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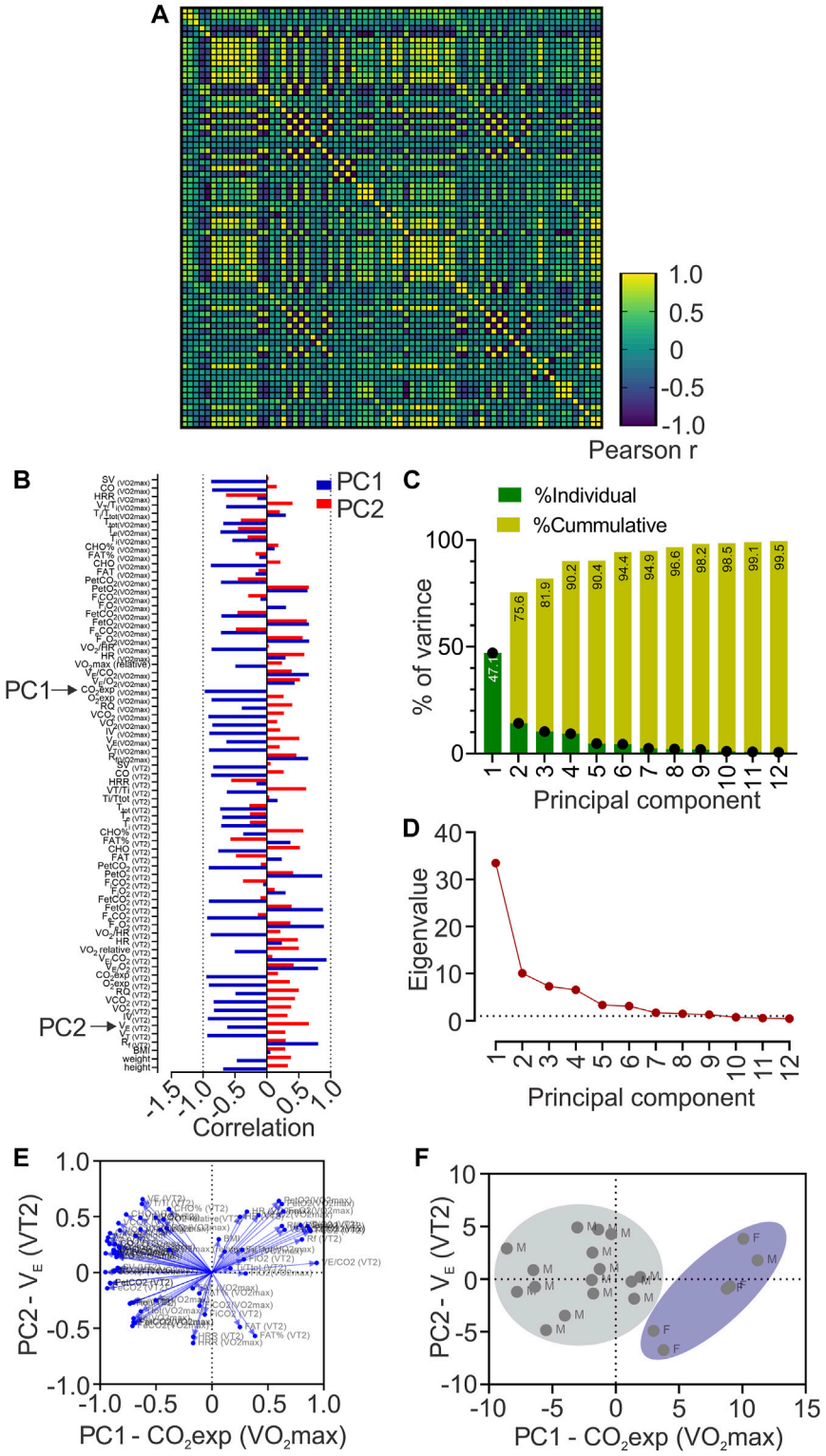

FIGURE 2
Principal components analysis (PCA). **(A)**, Heatmap of Pearson correlation coefficients between all data sets showing Pearson r values; **(B)**, Loadings of each to PC1 and PC2; **(C)**, Contribution of each Principal Component (PC) to the total variance of data; **(D)** Principal component analysis showing Eigenvalue higher than one in PC1 to PC6; **(E)**, Biplots are representing the contribution of each variable to the six first principal components; **(F)**, Biplot of plotted principal components scores.

in a non-athlete population. Then, our data suggest that these sex-related differences could be transversal, independent of the training regimen.

The VE/VO2 values have been previously used to describe the oxygen efficiency uptake (OUE) since OUE = 1000/VE/VO2 (Sun et al., 2012a; Sun et al., 2012b). The oxygen efficiency uptake plateau (OUEP) corresponds to a 90-s average of the highest consecutive measurements from VO2/VE (Sun et al., 2012a; Sun et al., 2012b). Sun and colleagues (2012) showed that OUEP is a good predictor of early death in patients with heart failure (Sun et al., 2012b). Further, the oxygen uptake efficiency at the anaerobic threshold, which can be obtained from a 60-s average of OUE, was similar and highly associated with OUEP (Sun et al., 2012a; Sun et al., 2012b; Sheridan et al., 2021). Nevertheless, OUEP does not accurately predicts VO2max in non-athletes (Sheridan et al., 2021). Likewise, a recent study reported no correlation between OUEP and VO2max in runners even after an increase in OUE (Jost et al., 2022). Despite the previously mentioned absence of correlation between OUEP, OUE at anaerobic threshold (or other values through VE/VO2 signal) with performance variables, such as VO2max, physiologically VO2 as well as VE both are influenced by cardiorespiratory and peripheral variables (Sun et al., 2012a; Sun et al., 2012b) Therefore, we used PCA to show how COP, a simplified index to obtained from the lowest VE/VO2 ratio in 60 s, influences metabolic and cardiorespiratory variables at VO2max and VT2 in the next section.

Contribution of principal components to COP in endurance athletes

During a CPET, there are several measures and calculated variables (cardiorespiratory and metabolic); however, despite that this would be considered an advantage, at the same time, it could also generate confusion. Currently, we evaluate 72 variables (cardiorespiratory and metabolic) derived from CPET, and we used PCA to determine what variables were more related to COP variance. PCA revealed that PC1 and PC2 explain 75.6% of the total COP variance. The principal variables that mainly explain the PC1 and PC2 were CO_2exp at VO2max and VE at VT2, respectively. Mechanistically, during CPET, the CO_2 production is related to metabolic acidosis, which is compensated by hyperventilation, reflected by an increase of VE (Nicolò et al., 2020a). Our data revealed that CO_2exp at VO2max mainly explains the COP variance (PC1: 47.1%); however, we found that CO_2exp at VO2max has shown a negative correlation with COP. Accordingly, this negative association could potentially support that lower COP allows better metabolic compensation at VO2max and could be relevant considering that world-class skiers showed two times a longer plateau at VO2max compared to national-class skiers (Sandbakk et al., 2011). Besides, several cardiopulmonary variables relevant to reaching high VO2max in endurance athletes are loading PC1 (Figure 2B) (Midgley et al., 2007). Indeed, VO2max is mainly limited by the stroke volume (SV) in well-trained athletes (Ouellet et al., 1969). Then, it is possible to propose that a lower COP value could be related to a better performance (negatively correlated with SV at VO2max). In addition, despite the lower number of female participants, we found that female subjects formed a cluster, which reflects that CO_2exp at VO2max (PC1), could be more relevant to females than male participants to explain the COP variance.

Principal component results revealed that PC2 explained 28.5% of total COP variance, explained mainly by VE at VT2. In addition, we found that VE at VT2 was negatively correlated with COP. It has been proposed that ventilatory response to exercise is critical to maintaining endurance performance (Tiller, 2019; Nicolò et al., 2020a; Nicolò et al., 2020b). Indeed, elite endurance athletes reach <75% VO2max during long-term time-trial running, evidencing that running a marathon reaches moderate to high VE (Joyner and Coyle, 2008). Higher VE is necessary to maintain altered homeostasis when CO_2 production increases (Ghosh, 2004). Moreover, it has been evidenced that one of the training adaptations in elite cyclists results in an increased VE at VT2 (Hoogeveen, 2000). In our study, those athletes with lower COP reached higher VE at VT2. Hence, PCA revealed that a low COP could help predict better cardiopulmonary variables for higher performance. It is essential to mention that VE is influenced by VT and Rf (Nicolò et al., 2020a); Rf has been demonstrated to be significant in sports and exercise (Nicolò et al., 2020b), and has been related to muscle fatigue (Marcora et al., 2008), rate of perceived exertion, and exercise tolerance (Nicolò et al., 2014; Nicolò et al., 2016; Nicolò et al., 2018), while VT increases to match VE (Nicolò et al., 2014; Nicolò et al., 2016; Nicolò et al., 2017; Nicolò et al., 2018). Therefore, our result suggests that COP could be associated with performance-related variables, which in turn suggests that the assessment of COP, obtained at submaximal intensity, could contribute to determining the competitive state of the athletes, at a low time-effort ratio. COP can be assessed likely without the interference of intensity distribution training since elite, and world-class athletes' prominent characteristic is to perform at high volume at low-intensity (Sandbakk et al., 2011). In contrast, high intensity is prescribed at a lower volume to avoid chronic stress (Seiler, 2010; Stöggl and Sperlich, 2015). In addition, COP can possibly be helpful during short and long-term training cessation (i.e., injury) to estimate detraining. However, our study was not focused on injured athletes because first, we needed to find whether, on the same subject, the COP could be influenced by some performance-related variables. Then, in future research, it is crucial to determine the impact of short and/or long-term training cessation on COP and determine if it could help to predict how much the athlete has been affected, contributing to the return sport continuum in highly trained subjects. Finally, our findings can be useful in non-athlete population or with chronic disease, where COP assessment could help to determine which variables contained in PC1 and PC2 are being affected allowing clinicians to aim different approaches of treatment.

Strength and limitations

Our study is not without limitations. Our sample was healthy athletes and not injured athletes, which could limit the interpretation of the results. However, it is essential to mention that, first, we need to find whether, in the same subjects, the COP could be influenced by some performance-related variables after moving to injured athletes. Furthermore, no blood samples were taken, which could contribute to a better understanding of the relationship between metabolic demands and oxygen consumption efficiency related to COP. Then, our results from PCA strongly support that COP could be influenced by VT2 and VO2max cardiorespiratory and metabolic variables; thus, this may help to support the notion of using the COP as a possible tool to track ventilatory thresholds to delineate exercise intensity domain and

assess how cardiopulmonary and metabolic performance is affected by, i.e., short, and long-term training cessation.

In summary, PCA revealed that COP variance was mainly explained by CO_2exp at VO2max and VE at VT2. Hence, COP could be an index capable of evaluating O2 consumption efficiency and tracking ventilatory thresholds at different exercise intensity domains due to its association with cardiopulmonary and metabolic variables, as well as performance-related variables. Thus, considering that COP can be obtained at submaximal intensity, our results strongly suggest that the COP calculation in these athletes could be used as a time-effort efficiency evaluation and monitoring tool during the off-season and competitive periods, as well as through rehabilitation and the return to sport continuum.

Conclusion

Our data suggested that COP evaluation could provide crucial information about cardiorespiratory performance-related variables at VT2 and VO2max, allowing us to identify an area of improvement among described PC variables. Besides, exercise performance can be negatively affected due to short-term and long-term stop training, i.e., off-season, illness, injuries, or surgical procedures. Thus, COP could be helpful to track ventilatory thresholds or even be implemented in early rehabilitation to estimate the current cardiorespiratory performance of the athlete and their evolution during the sports reinstatement process without the need for a maximal test (i.e., CPET). In addition, PCA revealed that COP is closely related to cardiopulmonary variables at VT2 and VO2max, which supports its usefulness in determining actual performance.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical committee of Universidad Mayor (#169_2019). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AO-A and AA-A performed data collection and analysis, and performed interpretation of the data. AO-A drafted the manuscript. AA-A, CS-A, RR-C, CA, CT, and MI, contributed to the interpretation of the data and preparation of the manuscript. DA contributed to the concept of the project. DA performed the interpretation of the data and contributed to the preparation of the manuscript. All data analysis and interpretation were undertaken in the laboratory of DA. All authors approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1087829/full#supplementary-material

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Glossary

CPET Cardiopulmonary exercise test
COP Cardiorespiratory optimal point
VO2 Oxygen consumption
VCO_2 Carbon dioxide output
VE Minute ventilation
VE/VO2 Minute ventilation to oxygen consumption ratio
PCA Principal Component Analysis
VO2max maximal oxygen consumption
VT ventilatory thresholds
VT1 Ventilatory threshold 1
VT2 Ventilatory threshold 2
PC Principal component
PC1 expired CO_2 at VO2max
PC2 VE at VT2
FEV1 Forced expiratory volume at first second
VC Vital capacity
ECG Electrocardiogram
PEF Peak expiratory flow
PIF Peak inspiratory flow
FEF 25 Forced expiratory flow at 25%
FEF 50 Forced expiratory flow at 50%
FEF75 Forced expiratory flow at 75%
W Watts
O2 Oxygen
CO_2 Carbon dioxide
N2 Nitrogen
FVC Forced vital capacity
CO_2exp Expired CO_2

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Reliability of pathophysiological markers reflective of exercise-induced gastrointestinal syndrome (EIGS) in response to 2-h high-intensity interval exercise: A comprehensive methodological efficacy exploration

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The study aimed to determine the test-retest reliability of exercise-induced gastrointestinal syndrome (EIGS) biomarkers, and assess the association of pre-exercise short chain fatty acid (SCFA) concentration with these biomarkers in response to prolonged strenuous exercise. Thirty-four participants completed 2 h of high-intensity interval training (HIIT) on two separate occasions with at least 5-days washout. Blood samples were collected pre- and post-exercise, and analysed for biomarkers associated with EIGS [i.e., cortisol, intestinal fatty-acid binding protein (I-FABP), sCD14, lipopolysaccharide binding protein (LBP), leukocyte counts, *in-vitro* neutrophil function, and systemic inflammatory cytokine profile]. Fecal samples were collected pre-exercise on both occasions. In plasma and fecal samples, bacterial DNA concentration was determined by fluorometer quantification, microbial taxonomy by 16S rRNA amplicon sequencing, and SCFA concentration by gas-chromatography. In response to exercise, 2 h of HIIT modestly perturbed biomarkers indicative of EIGS, including inducing bacteremia (i.e., quantity and diversity). Reliability analysis using comparative tests, Cohen's *d*, two-tailed correlation, and intraclass correlation coefficient (ICC) of resting biomarkers presented good-to-excellent for IL-1ra (r = 0.710, ICC = 0.92), IL-10 (r = 0.665, ICC = 0.73), cortisol (r = 0.870, ICC = 0.87), and LBP (r = 0.813, ICC = 0.76); moderate for total (r = 0.839, ICC = 0.44) and per cell (r = 0.749, ICC = 0.54) bacterially-stimulated elastase release, IL-1β (r = 0.625, ICC = 0.64), TNF-α (r = 0.523, ICC = 0.56), I-FABP (r = 0.411, ICC = 0.21), and sCD14 (r = 0.409, ICC = 0.38), plus fecal bacterial α-diversity; and poor for leukocyte (r = 0.327, ICC = 0.33) and neutrophil (r = 0.352, ICC = 0.32) counts. In addition, a medium negative correlation was observed between plasma butyrate and I-FABP (r = −0.390). The current data suggest a suite of biomarkers should be used to determine the incidence and severity of EIGS. Moreover, determination of plasma and/or fecal SCFA may provide some insight into the mechanistic aspects of EIGS instigation and magnitude in response to exercise.

KEYWORDS

running, I-FABP, endotoxin, bacteria, inflammation, gastrointestinal symptoms, short chain fatty acids

Introduction

It is well established that exposure to prolonged strenuous exercise creates a reversible disturbance to the gastrointestinal tract through two predominant pathways. Firstly, the circulatory-gastrointestinal pathway, which describes redistribution of blood flow from the gastrointestinal tract to working muscle and peripheral circulation, to aid locomotion work and thermoregulation, respectively. Secondly, the neuroendocrine-gastrointestinal pathway, which describes the resulting increase in sympathetic drive, and therefore, a reduction in gastrointestinal function (Costa et al., 2017b; Costa R. J. S. et al., 2020). The combination of these gastrointestinal disturbances in response to exercise stress have been termed as "*exercise-induced gastrointestinal syndrome*" (EIGS), and are often linked to exercise-associated gastrointestinal symptoms (Ex-GIS) [e.g., belching, bloating, upper and lower abdominal bloating or pain, urge to regurgitate or defecate, regurgitation, abnormal defecation (excessive watery stools), and/or nausea]; which may manifest into performance impairment and/or health issues warranting medical attention and/or management (Costa et al., 2017a; Gaskell et al., 2019; Gaskell et al., 2021a; Gaskell et al., 2021b; Walter et al., 2021). Various extrinsic and intrinsic factors have been identified to exacerbate EIGS and Ex-GIS, as previously described and updated (Gaskell et al., 2021a; Costa et al., 2022). However, in the majority of studies such observations are predominantly limited to a narrow number of gastrointestinal integrity and/or functional variables, with none to modest magnitude in perturbations due to the limited exercise stress load (Costa et al., 2022). More recently, the impact of a longer (2 h) duration high-intensity interval exercise protocol, prompted increases in plasma variables indicative of intestinal epithelial integrity perturbations [Δ pre-to post-exercise intestinal fatty acid binding protein (I-FABP) +737 pg/ml, and sCD14 + 110 ng/ml], Ex-GIS (62% incidence and 8 arb. unit severity), and malabsorption of post-exercise recovery beverages (Russo et al., 2021a; Russo et al., 2021b; Russo et al., 2021c). In comparison steady state exercise for the same duration appears to elicit a more modest response in these biomarkers [e.g., 2 h continuous running at 60% $\dot{V}O_2$ (I-FABP +447 pg/ml, gram-negative bacterial endotoxin +4 pg/ml, Ex-GIS 70% incidence and 6 arb. unit severity), and 70% $\dot{V}O_{2max}$ (I-FABP +371 pg/ml, sCD14 + 53 ng/ml, Ex-GIS 64% incidence and 16 arb. unit severity)] when performed in temperate ambient conditions (Snipe and Costa, 2018b; Costa et al., 2019).

It is however important to highlight that a large individual variation in biomarkers consistent with gastrointestinal integrity perturbations was observed. Therefore, to date, the reliability of these EIGS variables at rest and in response to exercise protocols established to perturb gastrointestinal integrity and/or cause functional disturbance (i.e., 2 h high intensity interval exercise), plus induce Ex-GIS, is still relatively unknown.

A potent secondary outcome of EIGS, caused by epithelial injury and/or hyperpermeability of the circulatory-gastrointestinal pathway, is the potential for luminal originated microbial pathogenic agents to translocate into systemic circulation (Gill et al., 2015a; Gill et al., 2015b; Gill et al., 2016a), which may lead to systemic inflammatory responses, with or without clinical implications (Peake et al., 2015). Although there is substantial research exploring the impact of exercise stress on direct or indirect markers of bacterial endotoxin translocation [e.g., plasma lipopolysaccharides (LPS), LBP, sCD14, and/or EndoCAb concentration], research into exercise-associated whole bacterial luminal to systemic translocation (i.e., bacteremia) is scarce (Costa et al., 2022). Some previous studies have attempted to detect whole bacteria presence in circulation (e.g., total 16S bacteria: *Bacteroides* ratio) in response to prolonged low intensity exercise (i.e., 80 min fixed-intensity treadmill walking- 6 km/h and 7% gradient) (Ogden et al., 2020a; Ogden et al., 2020b), and higher intensity of shorter duration exercise (i.e., 60 min running at 70% $\dot{V}O_{2peak}$) (March et al., 2019; Ogden et al., 2022), with findings modest and inconsistent. Variability of bacterial translocation in response to these exercise bouts may be explained by the application of insufficient exertional stress among studies, a lack of control of confounding factors within experimental procedures, and/or the biomarker selected to represent whole bacterial translocation into systemic circulation (i.e., *Bacteriodes*/total bacterial DNA) (Costa et al., 2022).

No study to date has determined whether whole bacteria luminal translocation occurs in response to a more substantial and relevant exercise stress model reflective of real-life practices in athletes who frequently report gastrointestinal issues (i.e., endurance and team sports) (Gaskell et al., 2021a; Gaskell et al., 2021b). Furthermore, no study has reported the full plasma bacterial composition profile at rest and in response to exercise stress, as previously reported in both the clinical and exercise research arena (Castillo et al., 2019; Villarroel et al., 2022).

Research investigating short-chain fatty acids (SCFA) (i.e., butyrate, acetate, and propionate) and the potentially protective role these may play in gastrointestinal epithelial integrity, systemic responses, and impact on exercise performance, is attracting interest and gaining momentum (Clauss et al., 2021; Imdad et al., 2022). It has been demonstrated, albeit *in-vitro,* that bathing epithelial cell lines in concentrated SCFA solutions, particularly with butyrate, reduces permeability of epithelial cells (Mariadason et al., 1997). Thus, SCFA may have potential to attenuate exercise-associated epithelial perturbations, and subsequent systemic endotoxemia and/or bacteremia, which may flow onto reducing microbial translocation associated Ex-GIS incidence and severity (Gill et al., 2015a; Gill et al., 2015b; Gaskell et al., 2021b). Such protection may be attributed to enhanced epithelial cell (i.e., phospholipid bilayer) stability and/or tight-junction stability and regulation (Sekirov et al., 2010; Gilbert et al., 2018; Nabizadeh et al., 2022). There is evidence in human-exercise models to hypothesise that the presence of SCFA along the intestinal lumen, as a result of commensal microbial composition and function, may attenuate the effects of EIGS (Bennett et al., 2020). However, to date, no study has investigated whether an association exists between pre-exercise

concentrations of luminal and systemic SCFAs and post-exercise markers of EIGS.

With this in mind, the current study primarily aimed to comprehensively determine the test-retest reliability of selected biomarkers linked to EIGS at rest prior to exercise and in response to prolonged strenuous exercise. In addition, a secondary aim was to assess the association between luminal and systemic SCFA concentration with these variables.

Methods

Participants

Thirty-four (n = 26 males, n = 8 females) recreationally competitive individuals exposed to endurance type training [mean (SD): Age 30 (8.0) years, nude body mass (NBM) 70.7 (10.3) kg, height 175 (9.0) cm, % body fat 15.9 (6.5) %, $\dot{V}O_{2max}$ 54.8 (5.6) ml/kg BM/min], volunteered to participate in the study. All participants gave written informed consent. The study protocol received approval from the Monash University Human Research Ethics Committee (MUHREC: 12799) and conformed with the 2008 Helsinki Declaration for Human Research Ethics. Standard exclusion criteria were applied as previously described (Costa et al., 2017a). Data presented within is additional follow-on data analysis from previous original experimental research (Russo et al., 2021a; Russo et al., 2021b; Russo et al., 2021c), registered with the Australian and New Zealand Clinical Trials Register (ANZCTR reference number 375090).

Preliminary measures

One to 3 weeks prior to the first experiment trial, baseline measurements for height (Stadiometer, Holtain Limited, Crosswell, Crymuch, United Kingdom), body mass (BM) (Seca 515 MBCA, Seca Group, Hamburg, Germany), body composition (Seca 515 MBCA, Seca Group, Hamburg, Germany) and $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, CA, United States) were recorded. $\dot{V}O_{2max}$ was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (Forma Run 500, Technogym, Seattle, WA, United States), as previously reported (Costa et al., 2009). Criteria for attaining $\dot{V}O_{2max}$ included participant reaching volitational exhaustion [i.e., rating of perceived exertion (RPE) of 19-20 Borg scale], a heart rate (HR) within 10 beats/min of HR_{max}, with observation of $\dot{V}O_2$ plateau in increasing exercise intensity and/or inclusion of RER (1.100). To determine running speeds for the exercise trials, the speed at approximately 50 [mean (SD): 7.3 (1.0) km/h], 55-60 [8.7 (1.3) km/h], 70-75 [10.8 (1.4) km/h], and 80-85 [12.7 (1.8) km/h] % $\dot{V}O_{2max}$ and 1% gradient was determined and verified from the $\dot{V}O_2$-work rate relationship.

Experimental procedures

A schematic illustration of the experimental procedures is depicted in Figure 1. Participants undertook two exertional-stress experimental protocols. To accommodate participant availability, washout ranged from 5-days to 14-days washout (mean 10-days). Considering the potential impact of dietary intake on the measured gastrointestinal variable in response to exercise (Costa et al., 2016), participants were provided with a standard low FODMAP diet the day before the experimental trials [energy 10.1 (3.0) MJ/day, protein 98 (30) g/day, fat 57 (36) g/day, carbohydrate 353 (87) g/day, fiber 44 (11) g/day, water 2.3 (1.4) L/day, <5 g/day FODMAP]. Dietary compliance was assessed using a food-fluid dietary log and compliance checklist. In addition, participants were asked to avoid alcohol and strenuous exercise for 48 h prior, and avoid consumption of caffeinated beverages 24 h prior to each experimental trial. Trials for female athletes were scheduled during the follicular phase of their menstrual cycle or when taking the active medication of oral contraceptive pill. Resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured for verification and were within normal range for both trials (<20.0 pg/ml).

On the day of experimental trials, the participants reported to the laboratory at 0800 h after consumption of a standardised low FODMAP breakfast [energy 2.9 (0.8) MJ, protein 28 (9) g, fat 19 (5) g, carbohydrate 99 (28) g, fiber 12 (5) g, and water 363 (264) ml] at 0700 h. From arrival to the lab until the initiation of exercise, participants were asked to void, and in addition, provide a 30 g mid-flow fecal sample (n = 27) collected in a sterilised fecal collection container (SARSTEDT Australia Pty Ltd, Mawson Lakes, South Australia, Australia). Fecal samples were immediately stored frozen at −80°C until sample processing and analysis. Nude BM and total body water (TBW) (Seca 515 MBCA, Seca Group, Hamburg, Germany) were then recorded prior to exercise. A breath sample was taken using 250 ml collection bag (Wagner Analysen Tecknik, Bremen, Germany) and blood samples collected by venepuncture from an antecubital vein into two separate vacutainers (6 ml 1.5 IU/ml lithium heparin and 4 ml 1.6 mg/ml K_3EDTA; BD, Oxford, United Kingdom). Participants completed an exercise-specific modified visual analog scale (mVAS) GIS assessment tool (Gaskell et al., 2019) and prior to starting exercise, inserted a thermocouple 12 cm beyond the external anal sphincter to record pre-exercise rectal temperature (T_{re}) (Precision Temperature 4600 Thermometer, Alpha Technics, CA, United States).

The exercise protocol began at 0900 h and consisted of 2 h high intensity interval (HIIT) running in T_{amb} 23.0 (1.0)°C and 43.5 (7.5)% relative humidity (RH) (Figure 1). Participants completed 6 × 20-min circuits consisting of 3 × 5-min of running at varying intensities (210 s at 50%–60% $\dot{V}O_{2max}$, 60 s at 65%–70% $\dot{V}O_{2max}$ and 30 s at 80%–85% $\dot{V}O_{2max}$) for a total of 15-min followed by 20 plyometric drop jumps and then walking at 6 km/h for the remaining 5-min of the 20-min cycle. The protocol was established based on exercise stress models that perturb immune and gastrointestinal status compared with baseline (Costa et al., 2009; Costa et al., 2011; Snipe and Costa, 2018a; Snipe and Costa, 2018b; Costa et al., 2019; Russo et al., 2019; Costa R. J. S. et al., 2020). Participants were provided with water equivalent to 3 ml/kgBM/h during exercise (Costa et al., 2009, Costa et al., 2011). Measures of HR (Polar Electro, Kempele, Finland), RPE and McGinnis 13-point thermal comfort rating scale (TCR) were taken during exercise at the 15-min mark of every 20-min cycle (Costa et al., 2014), and HR and

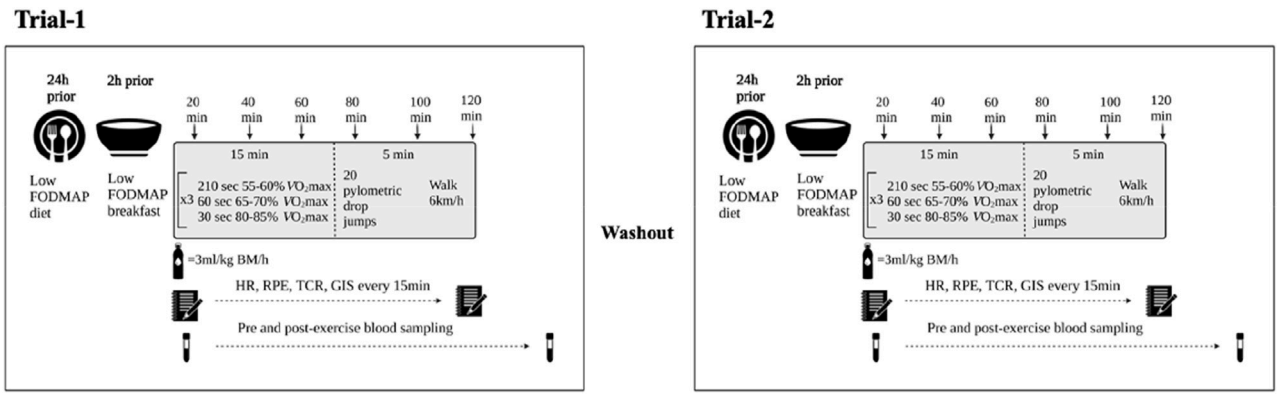

FIGURE 1
Schematic illustration of experimental procedures.

Ex-GIS were measured during the final 30 s of a 20-min cycle. Immediately post-exercise, nude BM and T_{re} were recorded. Blood samples were then collected 30 min into recovery as part of the primary recovery intervention research (Russo et al., 2021a; Russo et al., 2021b; Russo et al., 2021c). Breath samples and GIS were recorded every 30 min throughout the 2 h recovery period.

Blood sample analysis

The HemoCue system (Glucose 201+, Hb201, and WBC DIFF, HemoCue AB, Angelholm, Sweden) was used to determine blood glucose concentration, haemoglobin, total and differential leukocyte counts (including neutrophils, lymphocytes and monocytes) in duplicate from whole blood samples. The coefficient of variation (CV) for blood glucose concentration, haemoglobin and leukocyte counts was 5.1%, 1.6%, and 13.6% respectively. Haematocrit was determined by capillary method in triplicate from heparin whole blood samples using a microhematocrit reader (CV: 0.7%) (Thermo Fisher Scientific). The haemoglobin and haematocrit values were used to determine changes in plasma volume (P_v) relative to baseline, and to correct plasma variables (Dill and Costill, 1974). Bacterially-stimulated elastase release was determined as previously described (Costa et al., 2009; Costa et al., 2011; Costa et al., 2019a; Costa R. J. S. et al., 2020). The remaining whole blood in the heparin and K3EDTA vacutainers was centrifuged at 4,000 rpm (1,500 g) for 10 min within 15 min of sample collection and aspirated into 1.5 ml micro-storage tubes and frozen at −80°C until analysis. Prior to freezing, two 50 µl aliquots of heparin plasma were used to determine plasma osmolality (P_{Osmol}), in duplicate (CV: 0.7%), by freeze point osmometry (Osmomat 030, Gonotec, Berlin, Germany). Circulating concentrations of cortisol (DKO001; DiaMetra, Italy), PMN elastase (BMS269; Affymetrix EBioscience, Vienna, Austria), I-FABP (HK406; Hycult Biotech, Uden, Netherlands), sCD14 (HK320; Hycult Biotech), and LBP (HK315, Hycult Biotech) were determined by ELISA. Additionally, systemic cytokine profile [i.e., plasma IL-1β, TNF-α, IL-10, and IL-1ra concentrations] (HCYTMAG-60K, EMD Millipore, Darmstadt, Germany) were determined by multiplex system (Magpix, Luminex, Austin, TX, United States). All variables were analysed as per manufacturer's instructions on the same day, with standards and controls on each plate, and sample from each participant assayed on the same plate. The intra- and inter-assay CV for analysed biomarkers, respectively, was 6.1% and 10.4% for cortisol, 2.8% and 3.6% for I-FABP, 4.0% and 9.3% for LBP, 3.3% and 4.2% for sCD14, 5.5% and 9.7% for elastase, 16.0% and 16.6% for IL-1β, 14.9% and 15.5% for TNFα, 15.8% and 9.1% for IL-6, 14.7% and 12.6% for IL-8, 15.9% and 11.1% for IL-10, and 9.2% and 8.8% for IL-1ra.

Fecal and plasma microbial profiling

Upon thawing at room temperature, fecal samples were homogenised, then 0.20–0.30 g of each sample were transferred to a 2 ml dry garnet bead microtube, before the addition of bead solution. Cell lysis, sample purification, and DNA extraction was then performed as per manufacturer's instructions (PowerFecal DNA isolation kit, MoBio Laboratories, Qiagen, Germantown, United States). Blank control samples, using pyrogen/DNAse/RNAse free water in replacement of biological sample, were run simultaneously in duplicate. Purified extracted DNA (50 µl sample) was immediately frozen at −20°C prior to bacterial gene sequencing. Extracted genomic DNA was delivered to the Australian Genome Research Facility (Melbourne, Australia) for PCR amplification of the V3-V4 region of the 16S rRNA gene, and sequencing on the Illumina MiSeq platform utilising the Illumina's Nextera XT Index kit. Blank control samples yielded undetectable outcomes.

For detection and profiling of bacterial content in plasma samples a QIAamp UPC pathogen mini kit was used to extract microbial DNA (MoBio Laboratories, Qiagen, Germantown, United States), prior to s16 gene sequencing. After thawing, 200 µl of heparin plasma was added to 1.5 ml glass microbead tubes and underwent mechanical lysis in accordance with manufacturer's instructions. Thereafter, 400 µl of the pre-treated sample underwent the spin protocol for chemical lysis in accordance with the manufacture's instruction. Purified extracted DNA (100 µl sample) was immediately frozen at −20°C prior to bacterial gene

sequencing. Blank control samples, using pyrogen/DNAse/RNAse free water in replacement of biological sample, were run simultaneously in duplicate. Extracted genomic DNA was delivered to the Micromon Next-Generation Sequencing Facility (Monash University, Clayton, Australia) for PCR amplification of the V3-V4 region of the 16S rRNA gene as previously described (Bennett et al., 2020). Prior to s16 gene sequencing, microbial DNA detection and concentration was performed by Qubit fluorometer quantification (ThermoFisher Scientific, Waltham, MA, United States) in duplicate (CV: 2.5%). Blank control samples yielded undetectable outcomes.

The assembled reads were analysed using QIIME2 (v.2019.1), as previously described (Bennett et al., 2020). Briefly, reads were imported into QIIME2 with quality assessment, filtering, barcode trimming, and chimera detection were performed using the DADA2 pipeline. Taxonomic evaluation using pre-set parameters (98% identify, confidence p <0.05%) with the SILVA 138.1 release (Quast et al., 2013). Sequencing data are available on the Short Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra), BioProject number PRJNA926792. Before statistical analysis, sequencing data for phyla, family, and genus amplicon sequence variants were calculated by dividing the number of reads for each taxon by the number of reads in the fecal and plasma samples.16S rRNA sequences per fecal and plasma samples ranged from 33,197 to 219,089 and 13,091 to 42,715, respectively. To minimise the risk of including artefact values in data analysis, resulting from potential contamination during sample handling (i.e., sample collection, processing and analysis procedures), a fresh sample was collected and immediately processed and frozen. The samples were processed in a sterile laboratory and UV biological grade fume cabinet (Safemate 1.2 ECO, LAF Technologies Pty Ltd, Baywater North, Victoria, Australia) and pyrogen/DNAse/RNAse free sample processing consumables were used. For amplicon sequence variants (AVS), only bacterial groups with a conservative ≥0.5% relative abundance, respective to the determination medium, were included for data analysis (Nikkari et al., 2001; Luo et al., 2021).

Bacterial calculations of n = 5 and n = 5 phyla, n = 22 and n = 28 family, n = 40 and n = 22 genus AVS, for fecal and plasma samples respectively, were adequately detected for relative abundance and α-diversity (i.e., Shannon Index (SI) and Shannon Equitability Index (SEI) determination.

Fecal and plasma short chain fatty acid (SCFA) analysis

Fecal SCFAs were measured by gas chromatography as previously described (Clarke et al., 2011). Thawed fecal material was spiked with three times the volume of internal standard (1.68 mM heptanoic acid), homogenized and centrifuged (2000 g, 10 min, 4°C). After centrifugation, 300 µl of supernatant was added to a 0.2 µm filter vial containing 10 µl of 1 M phosphoric acid. The vials were then analysed for SCFA content *via* gas chromatography. Samples were analysed using an Agilent GC6890 gas chromatography coupled to a flame-ionisation detector (FID), with helium used as the carrier gas. An Agilent free fatty acid phase (FFAP) column (30 m × 0.53 mM (internal diameter) x 1.00 µM (film thickness) was installed for analysis. A splitless injection technique was used, with 0.2 µl of sample injected. A constant flow rate of 4.0 ml/min was used on the column. Upon injection, the oven was initially held at 90°C for 1 min, then raised to 190°C at 20°C/min and held for 3 min. Samples were run in triplicate to ensure accurate and replicable data were obtained. A CV <10% within triplicate samples was used as a quality control measure.

Plasma samples (heparin) were analysed in duplicate for SCFA content using gas-chromatography, as previously described (Gill et al., 2020). Briefly, 300 µl of plasma was spiked with 50 µl of 200 µM heptanoic acid and acidified with the addition of 50 µl of 10% sulfosalicylic acid before the addition of 3 ml diethyl ether solvent. The mixture was vortexed and centrifuged so that the organic layer could be clarified and transferred into 50 µl 0.2 M NaOH. The alkaline solution containing SCFA was concentrated by evaporation using nitrogen, dissolved in 30 µl 1 M phosphoric acid and transferred into a cold GC glass vial for analysis using an Agilent GC6890 coupled to FID. Concentrations for acetate, propionate and butyrate were determined by the average of the triplicate results, where the CV was <20%. Total SCFA was calculated by the sum of the individual SCFA. Results were expressed as µmol/L.

Statistical analysis

Of the original metadata collection (Russo et al., 2021a; Russo et al., 2021b; Russo et al., 2021c), confirmation of adequate statistical power was determined *a priori* for EIGS biomarkers (i.e., circulating leukocytes, and plasma concentration of I-FABP, bacterial endotoxins, and inflammatory cytokines) and Ex-GIS by applying the mean, standard deviation, and effect size on these variable in response to exertional stress (Costa et al., 2009; Costa et al., 2011; Costa et al., 2017a; Snipe and Costa, 2018a; Snipe and Costa, 2018b; Costa et al., 2019; Costa R. J. S. et al., 2020). Using G Power software, and applying a standard alpha (0.05) and beta value (0.80), the current participant sample size is estimated to provide adequate statistical power (power* 0.80-0.99) for detecting if there are any significant differences between the test-to-retest EIGS biomarker values (G_Power 3.1, Kiel, Germany), which was confirmed using *post hoc* software test application. Statistics were analysed using SPSS statistical software (V.27.0, IBM SPSS Statistics, IBM Corp., Armonk, NY, United States) with significance accepted at $p \leq 0.05$. Data in text and tables are presented as mean and 95% confidence interval (CI) for variables. Intraclass correlation coefficient (ICC) was used for test-retest reliability analysis whereby ICC (absolute agreement, 2-way random effects model) range $r^a < 0.50$, $r^a = 0.50$-0.74, $r^a = 0.75$-0.90, and $r^a > 0.90$ was interpreted as poor, moderate, good and excellent, respectively (Koo & Li, 2016). Standard error of mean (SEM) was calculated using established formula: $SEM = SD \times \sqrt{1} - \text{ICC}$ and minimal detectable change (MDC) was calculated using previously established formula: $MDC = SEM \times 1.96 \times \sqrt{2}$ (Weir, 2005). Significance of test-retest variation was determined by the Wilcoxon signed-rank test. Magnitude of test-retest variation was determined by Cohen's d standardized measurement of effect size, whereby $d < .20$, $d = .20$ to .49, $d = .50$ to .80, and $d > .80$ for no, small, medium, and large effects, respectively. Test-retest correlation and agreement were assessed using Bland–Altman plots calculating bias and limits of agreement, as well as Spearman's correlation coefficients (whereby

TABLE 1 Indices of physiological strain measured during Trial-1 and Trial-2 in response to 2 h high intensity interval exercise in temperate ambient conditions.

	Trial-1	Trial-2	p
Mean heart rate- low pace (bpm)	125 (121–130)	120 (116–125)	**<.001**
Mean heart rate- high pace (bpm)	160 (156–165)	157 (152–162)	**.005**
Cardiac drift—low pace (bpm)	14 (11–16)	14 (11–17)	.750
Cardiac drift—high pace (bpm)	10 (8–12)	9 (7–10)	.214
Mean rating of perceived exertion	13 (13–14)	12 (12–13)	**<.001**
Δ rating of perceived exertion[a]	3 (3–4)	3 (3–4)	.781
Post-exercise T_{re} (°C)	37.9 (37.6–38.2)	37.8 (37.6–38.1)	.810
Δ T_{re} (°C)[b]	1.2 (0.9–1.6)	1.2 (0.9–1.4)	.795
Mean thermal comfort rating	9 (8–9)	9 (8–9)	.283
Δ thermal comfort rating[a]	2 (1–2)	1 (1–1)	.146

Mean and 95% CI (n = 34).
[a]Change from cycle 1 to cycle 6, and pre-to post-exercise change.

r_s< 0.300, r_s = 0.300 to 0.500, and r_s> 0.500 for weak, moderate, and strong, respectively). Limits of agreement were defined as mean bias ± 2 SD.

Results

Physiological strain

The indices of physiological strain measured on Trial-1 and Trial-2 are reported in Table 1. Pre- and post-exercise P_{Osmol} was 291 (288–294) and 293 (290–296) mOsmol/kg, respectively, and was not significantly different between trials (p = 0.052 and p = 0.546, respectively). Exercise-induced body mass loss and Pv change was 1.9 (1.7–2.1) % and −2.1 (−3.8 to −0.6) %, respectively, and was not significantly different between trials (p = 0.485 and p = 0.998, respectively). No difference in resting pre-exercise and post-exercise plasma cortisol concentration was observed between trials (Table 2). However, a significant increase in pre-to post-exercise plasma cortisol concentration was observed on Trial-1 only, but no substantial difference in magnitude of response was observed between the two trials (Table 3). Assessment of test-retest presented a strong correlation for resting pre-exercise plasma cortisol concentration, with good test-retest reliability (Figure 2A; Table 4), and a moderate correlation (r = 0.455) with poor test-retest reliability (ICC = 0.41) for exercise-associated change in plasma cortisol concentration (Supplementary Table S1; Figure 1).

Intestinal integrity

No difference in resting pre-exercise and post-exercise plasma I-FABP, sCD14 or LBP concentration was observed between trials (Table 2). Plasma I-FABP concentration increased in response to HIIT in Trial-1 and Trial-2, whereas plasma sCD14 and LBP concentration did not significantly change in either trial (Table 2). The magnitude of response for these biomarkers did not significantly differ between trials (Table 3). Assessment of test–retest presented a strong correlation with good test-retest reliability for resting pre-exercise plasma LBP concentration, and a moderate correlation with poor test-retest reliability for plasma I-FABP and sCD14 (Figures 2B–D, respectively; Table 4). The correlation for exercise-associated change between trials was moderate for plasma I-FABP concentration, and weak for plasma sCD14 concentration and LBP concentration (r = 0.319, r = −0.079, and r = 0.119, respectively) while test-retest reliability was poor for all three biomarkers (ICC ≤0.21) (Supplementary Table S1; Figure 1).

Leukocyte trafficking and bacterially-stimulated neutrophil degranulation

No difference in resting pre- and post-exercise total leukocyte and neutrophil counts were observed between trials (Table 2). Total leukocyte and neutrophil counts increased in response to HIIT in both Trial-1 and Trial-2 (Table 2). No difference in magnitude of response was observed between the two trials for these blood cell counts (Table 3). Assessment of test-retest correlation for resting total leukocyte and neutrophil counts was moderate with poor test-retest reliability (Figures 2E, F; Table 4), while exercise-associated change in total leukocyte and neutrophil counts presented moderate correlations (r = 0.487 and r = 0.468, respectively) with poor test-retest reliability (ICC ≤0.48) (Supplementary Table S1; Figure 1).

There was no difference in resting pre- and post-exercise total bacterially-stimulated elastase and elastase release per cell between trials (Table 2). The concentration of total bacterially-stimulated elastase increased in response to HIIT in both Trial-1 and Trial-2. No significant change was observed in elastase release per cell in either trial (Table 2). The magnitude of response for these *in-vitro* immune function biomarkers did not significantly differ between trials (Table 3). Assessment of test-retest revealed a strong correlation for resting plasma total bacterially-stimulated elastase (Figure 2G), but poor-to-moderate test-retest reliability (Table 4). Exercise-associated change from pre-to post-exercise for plasma total bacterially-stimulated elastase presented a strong correlation (r = 0.646), with good test-retest reliability (ICC = 0.72). A strong pre-exercise correlation, but moderate test-retest reliability (Figure 2H; Table 4), was presented for plasma elastase release per cell, with no correlation (r = 0.073) and poor test-retest reliability observed for exercise-associated change in elastase release per cell (ICC = 0.23) (Supplementary Table S1; Figure 1).

Systemic inflammatory cytokine profile

No difference in resting pre- and post-exercise plasma concentrations of IL-1β, TNF-α, IL-10 or IL-1ra was observed between trials (Table 2). An increase in plasma concentration pre-to post-exercise of anti-inflammatory cytokines, IL-10 and IL-1ra, was observed in both Trial-1 and Trial-2, while no other

TABLE 2 Pre- and post-exercise exercise-induced gastrointestinal syndrome (EIGS) biomarkers in response to 2 h high intensity interval exercise in temperate ambient conditions performed on two separate occasions.

	Pre Trial-1	Pre Trial-2	*d*	Post Trial-1	Post Trial-2	*d*
Cortisol (nMol/L)	603 (502–705)	598 (487–709)	.00	831 (692–970)**	716 (566–866)	.23
IFABP (pg/ml)	614 (444–783)	498 (400–596)	.22	1275 (940–1610)**	1136 (865–1406)**	.13
sCD14 (μg/ml)	2.4 (2.2–2.5)	2.5 (2.4–2.5)	.30	2.3 (2.2–2.4)	2.5 (2.3–2.6)	.40
LBP (μg/ml)	13.5 (11.2–15.8)	12.9 (10.8–15.0)	.08	14.4 (12.0–16.8)	13.4 (11.0–15.8)	.13
Total leukocyte counts (x10^9/L)	5.9 (5.3–6.5)	5.7 (5.0–6.4)	.11	8.0 (7.1–8.9)**	7.2 (6.5–7.9)**	.29
Neutrophil counts (x10^9/L)	3.3 (2.7–3.8)	3.2 (2.6–3.8)	.03	5.1 (4.3–5.9)**	4.4 (3.8–4.9)**	.27
Total stimulated elastase (ng/ml)	2217 (1351–3083)	3745 (2262–5227)	.40	3739 (2402–5076)**	4938 (3382–6494)**	.25
Elastase release per cell (fg/ml)	749 (379–1119)	650 (363–937)	.08	350 (236–464)	588 (363–836)	.44
IL-1β (pg/ml)	2.2 (1.2–3.1)	2.4 (1.2–3.7)	.07	2.0 (1.2–2.8)	2.6 (1.4–3.8)	.17
TNF-α (pg/ml)	9.6 (7.3–12.0)	10.3 (8.3–12.3)	.08	10.8 (8.4–13.1)	10.5 (8.4–12.3)	.03
IL-10 (pg/ml)	13.1 (7.9–18.3)	15.2 (9.2–21.2)	.12	27.4 (18.7–36.2)**	23.0 (16.7–29.3)**	.15
IL-1ra (pg/ml)	28.5 (18.9–38.1)	28.5 (19.8–37.2)	.00	33.7 (25.1–42.4)**	35.9 (26.0–45.7)**	.07
Plasma bacterial DNA (ng/μl)	0.03 (0.02–0.05)	0.03 (0.01–0.05)	.17	0.07 (0.06–0.08)**	0.07 (0.06–0.08)**	.07
Plasma phyla SEI	0.245 (0.213–0.276)	0.275 (0.252–0.294)	.44	0.217 (0.195–0.240)**	0.214 (0.195–0.233)**	.07
Plasma family SEI	0.292 (0.276–0.308)	0.295 (0.278–0.312)	.10	0.292 (0.278–0.305)	0.300 (0.284–0.316)	.25
Plasma genus SEI	0.244 (0.224–0.265)	0.242 (0.226–0.259)	.05	0.253 (0.239–0.267)	0.271 (0.260–0.282)**	.62

Mean and 95% CI. ** $p < 0.001$ *vs.* pre-exercise. Magnitude of test-retest variation was determined by Cohen's *d* standardized measurement of effect size, whereby $d < .20$, $d = .20$ to .49, $d = .50$ to .80, and $d > .80$ for no, small, medium, and large effects, respectively.

cytokines significantly changed in response to exercise in either trial (Table 2). A difference in magnitude of response of plasma IL-10 concentration was observed between Trial-1 and Trial-2 (Table 3). No substantial difference in magnitude of response was observed for any other systemic inflammatory cytokines. Assessment of test-retest presented a strong correlation for resting concentrations of all measured systemic inflammatory cytokines (Figures 2I–L). Test-retest reliability of resting plasma cytokine concentrations presented as excellent for IL-1ra, good for IL10, and moderate for IL-1β and TNF-α (Table 4). Weak correlations were observed between exercise-associated change for all systemic inflammatory cytokines, with the exception of plasma IL-10 concentration, which demonstrated a strong correlation between Trial-1 and Trial-2 ($r = 0.615$). All cytokines presented poor test-retest reliability for exercise-associated change in systemic inflammatory cytokine concentration (ICC ≤0.48) (Supplementary Table S1; Figure 1).

Pre-exercise fecal bacteria composition

At rest, the sufficient identification of relative abundance of bacterial phyla groups in fecal samples included: *Firmicutes* (69%), *Bacteroidota* (24%), *Actinobacteroita*, *Proteobacteria,* and *Verrucomicrobia* (2%), which did not substantially differ between trials ($p > 0.05$, $d \leq .46$). Resting fecal bacterial phyla SEI was 0.188 (95% CI: 0.166 to 0.211) and did not substantially differ between trials ($p = 0.098$, $d = .42$); but there was no significant Trial-1 *vs.* Trial-2 correlation observed (r = 0.325, $p = 0.237$) (Table 4).

At rest, the sufficient identification of relative abundance of bacterial family groups in fecal samples included: *Ruminococcaceae* (27%), *Lachnospiraceae* (27%), *Bacteriodaceae* (13%), *Acidaminococcaceae* (6%), *Prevotellaceae* (5%), *Christensenellaceae* (4%), *Veillonellaceae* (3%), *Rikenellaceae*, *Muribaculaceae*, *Akkermensiaceae*, *Pasteurellaceae*, and *Bifidobacteriaceae* (2%), and all other identified bacterial family groups (n = 10) at ≤1%. The relative abundance of bacterial family groups at rest did not substantially differ between trials ($p > 0.05$, $d \leq .47$), except for *Akkermensiaceae* (Trial-1 3.1% and Trial-2 0.6%; $p = 0.021$, $d = .46$). Resting fecal bacterial family SEI was 0.245 (95% CI: 0.234 to 0.256) and did not substantially differ between trials ($p = 0.440$, $d = .16$), with a significant Trial-1 *vs.* Trial-2 correlation observed (r = 0.554, $p = 0.032$) (Table 4).

At rest, the sufficient identification of relative abundance of bacterial genus groups in fecal samples included: *Bacteroides* (13%), *Faecalibacterium* (11%), *Agathobacter* (5.7%), *Phascolarctobacterium* (5.3%), *Prevotella 9* (4.3%), *Blautia* (4.2%), *Christensenellaceae R-7 group* (3.6%), *Roseburia* (3.5%), *Subdoligranulum* (3.2%), *Alistipes* (2.4%), *Veillonella* (2.1%), and *Eubacterium-coprostanoligenes group* (2.0%), and all other identified bacterial genus groups (n = 28) at ≤2%. The relative abundance of bacterial genus groups at rest did not significantly differ in the larger majority of detectable genera ($p > 0.05$, $d \leq .48$). Differences between Trial-1 and Trial-2 were only

TABLE 3 Pre-to post-exercise magnitude of change in exercise-induced gastrointestinal syndrome (EIGS) biomarkers in response to 2 h high intensity interval exercise in temperate ambient conditions performed on two separate occasions.

	Δ Trial-1	Δ Trial-2	*d*
Cortisol (nMol/L)	228 (99–357)	118 (−24–260)	.23
IFABP (pg/ml)	661 (357–964)	638 (390–885)	.02
sCD14 (μg/ml)	−0.02 (−0.12 to 0.08)	0.04 (−0.10–0.18)	.14
LBP (μg/ml)	0.91 (−0.20–2.00)	0.47 (−0.74–1.70)	.11
Total leukocyte counts (x10^9/L)	2.1 (1.3–2.9)	1.5 (0.6–2.3)	.22
Neutrophil counts (x10^9/L)	1.8 (1.1–2.6)	1.2 (0.6–1.8)	.26
Total stimulated elastase (ng/ml)	1522 (761–2283)	1193 (104–2283)	.12
Elastase release per cell (fg/ml)	−398 (−756 to −41)	−51 (−288 to 187)	.31
IL-1β (pg/ml)	−0.2 (−0.5 to 0.2)	0.2 (−0.5–0.8)	.18
TNF-α (pg/ml)	1.1 (−0.1–2.3)	0.2 (−1.2–1.6)	.21
IL-10 (pg/ml)	14.3 (6.6–22.0)	7.8 (3.8–11.8)[a]	.27
IL-1ra (pg/ml)	5.3 (0.7–9.8)	7.4 (4.0–10.7)	.14
Plasma bacterial DNA (ng/μl)	0.04 (0.02–0.05)	0.04 (0.02–0.07)	.19
Plasma phyla SEI	−0.03 (−0.06 to 0.00)	−0.06 (−0.09 to −0.03)	.41
Plasma family SEI	0.00 (−0.01 to 0.01)	0.00 (−0.02–0.03)	.13
Plasma genus SEI	0.01 (−0.01–0.03)	0.03 (0.02–0.04)	.46

Mean and 95% CI.

[a]$p < 0.05$ magnitude of response Trial-1 *vs.* Trial-2. Magnitude of test–retest variation was determined by Cohen's *d* standardized measurement of effect size, whereby $d < .20$, $d = .20$ to .49, $d = .50$ to .80, and $d > .80$ for no, small, medium, and large effects, respectively.

observed for *Akkermensia* (Trial-1 3.1% and Trial-2 at 0.6%; $p = 0.021$, $d = .46$), *Coprococcus-3* (Trial-1 0.5% and Trial-2 at 0.9%; $p = 0.045$, $d = .56$), *Dorea* (Trial-1 0.7% and Trial-2 at 1.3%; $p = 0.036$, $d = .73$), *Parabactercides* (Trial-1 0.9% and Trial-2 at 0.6%; $p = 0.040$, $d = .27$), and *Romboutsia* (Trial-1 0.3% and Trial-2 at 1.6%; $p = 0.046$, $d = .47$). Resting fecal bacterial genus SEI was 0.282 (95% CI: 0.269 to 0.296) and did not substantially differ between trials ($p = 0.632$, $d = .11$), with a significant Trial-1 *vs.* Trial-2 correlation observed (r = 0.585, $p = 0.022$) (Table 4).

Pre- and post-exercise plasma bacteria composition

Plasma bacterial DNA concentrations significantly increased from pre-to post-exercise in Trial-1 and Trial-2 ($p < 0.001$), with no difference between trials observed (Tables 2, 3). At rest, sufficient identification of relative abundance of bacterial phyla groups in plasma included: *Proteobacteria* (63%), *Firmicutes* (20%), *Actinobacteroita* (7%), *Bacteroidota* and *Cyanobacteria* (4%), which did not substantially differ between trials ($p > 0.05$, $d \leq .41$), except for *Bacteroidota* (2.8% trial difference; $p = 0.036$, $d = .65$). Resting plasma bacterial phyla SEI did not substantially differ between trials ($p = 0.032$, $d = .44$), with a significant Trial-1 *vs.* Trial-2 correlation observed (r = 0.607, $p = 0.016$). HIIT induced substantial changes in the plasma relative abundance of *Proteobacteria* (+7.9%), *Firmicutes* (−4.2%), and *Cyanobacteria* (−2.9%) ($p < 0.05$). HIIT-induced changes in plasma relative abundance of bacterial phyla did not differ between trials, except for *Bacteroidota* (5.6% trial difference; $p = 0.005$, $d = .96$). These changes translated into a significant reduction in pre-to post-exercise plasma bacterial phyla SEI ($p < 0.001$), which did not differ between trials (Tables 2, 3), and was associated with a correlation trend between Trial-1 *vs.* Trial-2 (r = 0.502, $p = 0.057$) (Table 3).

At rest, sufficient identification of relative abundance of bacterial family groups in plasma included: *Beicojerinckiaceae* (26%), *Halomonadaceae* (10%), *Bacillaceae* (7%), *Pseudomonadaceae* (7%), *Moraxellaceae* (6%), *Erysipelotrichaceae* (5%), *Chloroplast* (4%), *Sphingomonadaceae* (4%), *Lachnospiraceae* (3%), *Corynebacteriaceae*, *Xanthobacteraceae*, *Enterobacteriaceae*, *Comamonadaceae*, *Propionibacteriaceae*, *Chitinophagaceae*, and *Staphylococcaceae* (2%), and all other identified bacterial family groups (n = 12) at ≤1%, which did not substantially differ between trials ($p > 0.05$, $d \leq 0.75$), except for *Bacteroidaceae* (1.7% trial difference; $p = 0.008$, $d = .79$) and *Oxalobacteraceae* (0.6% trial difference; $p = 0.043$, $d = .54$). Resting plasma bacterial family SEI did not substantially differ between trials ($p = 0.752$, $d = .10$) (Table 2). HIIT induced substantial increases in the plasma relative abundance of *Proteobacteria* family groups *Sphingomonadaceae* (+5.8%), *Comamonadaceae* (+2.6%), *Oxalobacteraceae* (+1.2%), *Erwiniaceae* (+1.0%), and *Morganellaceae* (+0.5%); at the expense of reduced *Corynebacteriaceae* (−1.2%), *Bacteroidaceae* (−1.0%), *Chloroplast* (−3.1%), *Lachnospiraceae* (−2.4%), and *Ruminococcaceae* (−0.8%) ($p < 0.05$). These changes did not translate into a significant change in pre-to post-exercise plasma bacterial family SEI ($p = 0.716$), which did not differ between trials (Tables 2, 3).

t rest, sufficient identification of relative abundance of bacterial genus groups in plasma included: *Methylobacterium* (24%), *Halomonas* (10%), *Pseudomonas* (7%), *Anaerobacillus* (5%), *Acinetobacter* (5%), *Erysipelothrix* (5%), *Escherichia-Shigella* (1.9%), *Cutibacterium* (1.6%), *Sandarakinorhabdus* (1.6%), *Corynebacterium* (1.5%), *Bacillus* (1.4%), *Staphylococcus* (1.2%), *Sediminibacterium* (1.2%), and *Bacteroides* (1.2%), and all other identified bacterial genus groups (n = 8) at ≤1%, which did not substantially differ between trials ($p > 0.05$, $d \leq .47$), except for *Corynebacterium* (1.3% trial difference; $p = 0.011$, $d = .75$) and *Bacteroides* (1.8% trial difference; $p = 0.006$, $d = .86$). Resting plasma bacterial genus SEI did not substantially differ between trials ($p = 0.878$, $d = .05$) (Table 2). HIIT induced substantial increases in the plasma relative abundance of *Proteobacteria* genus groups *Sandarakinorhabdus* (+3.2%),

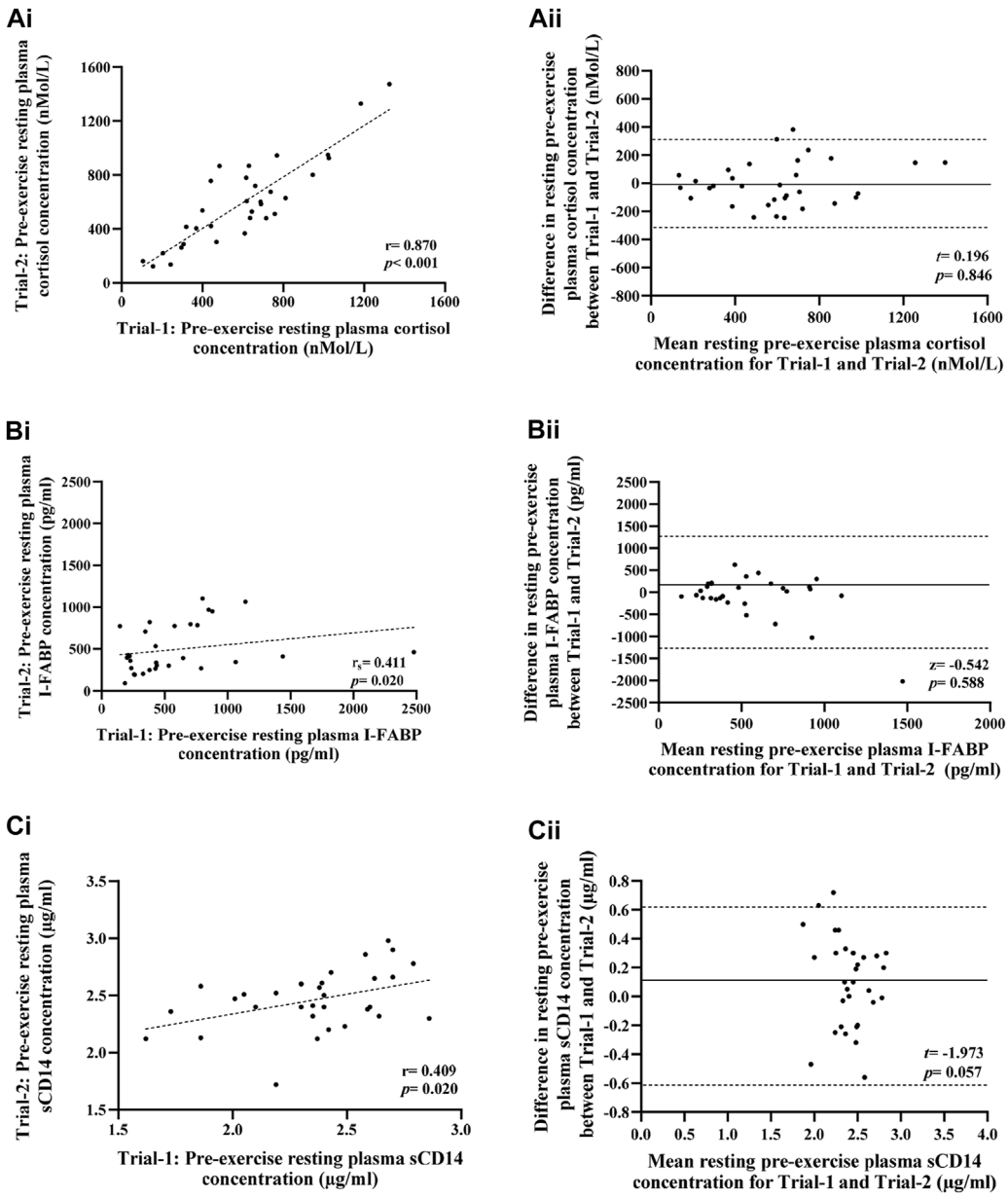

FIGURE 2
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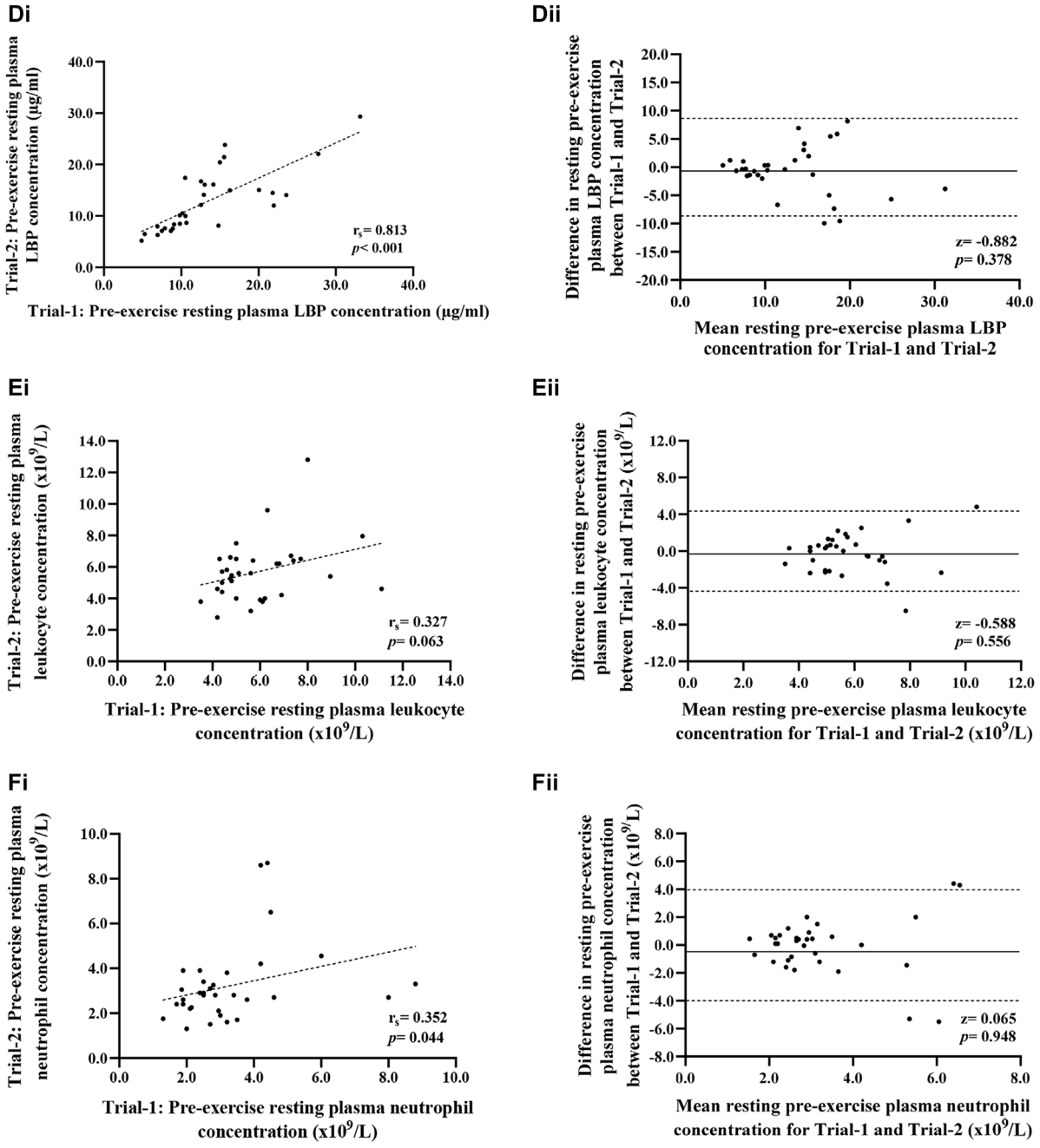
Sphingomonas (+1.6%), *Polaromonas* (+1.4%), *Massilia* (+1.3%), *Aquabacterium* (+0.6%), *Providencia* (+0.4%), and increased *Actinobacteriota* genus *Micrococcus* (+0.4%), at the expense of reduced *Bacteroidota* genus *Bacteroides* (−0.9%) ($p < 0.05$). These changes translated into a significant change in pre-to post-exercise plasma bacterial genus SEI ($p = 0.006$), which did not differ between trials (Tables 2, 3).

Fecal and plasma short-chain fatty acids (SCFA) concentration

No significant difference was observed between trials in pre-exercise resting total SCFA, acetate, propionate and butyrate fecal and plasma concentrations (Figure 3). Assessment of test-retest presented strong correlations for pre-exercise resting fecal total SCFA, acetate, valeric acid and caproic acid concentrations, and moderate correlations for fecal propionate and butyrate concentrations (Figure 3). Reliability analysis revealed moderate test-retest reliability for fecal measures of total SCFA, acetate and caproic acid concentrations, and poor test-retest reliability for fecal propionate, butyrate, and valeric acid (Table 4). Pre-exercise resting plasma concentrations of total SCFA and acetate concentrations presented moderate correlations ($r = 0.484$ and $r = 0.466$, respectively), while resting pre-exercise plasma propionate and butyrate concentrations presented weak correlations ($r = 0.176$ and $r = 0.273$, respectively). Reliability analysis revealed moderate reliability for pre-exercise total plasma SCFA, acetate,



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and butyrate concentrations, and poor reliability for pre-exercise plasma propionate (Table 4).

There was no significant relationship between pre-exercise fecal total and differential SCFA concentrations and the magnitude of change in I-FABP concentrations from pre-to post-exercise ($p > 0.05$). A moderate negative correlation was observed between plasma butyrate concentrations and I-FABP ($r = -0.390$ $p = 0.001$), with low levels of resting plasma butyrate concentration associated with increased plasma I-FABP concentrations post-exercise. A weak negative correlation was observed between pre-exercise plasma propionate concentrations and pre-to post-exercise concentrations of plasma IL-1ra ($r = -0.299$, $p = 0.015$) and pre-exercise fecal butyrate concentrations and pre-to post-exercise plasma cortisol ($r = -0.292$, $p = 0.044$).

Moderate positive correlations were observed between pre-exercise fecal total, fecal acetate and fecal caproic concentrations with plasma pro-inflammatory cytokine IL-1β ($r = 0.413$, $p = 0.004$, $r = 0.472$, p= <0.001 and $r = 0.411$, $p = 0.004$ respectively). Higher concentrations of fecal total, acetate and caproic pre-exercise were associated with a larger increase of IL-1β from pre-to post-exercise. A moderate positive correlation was observed between pre-exercise fecal acetate concentration and pre-to post-exercise plasma TNF-α

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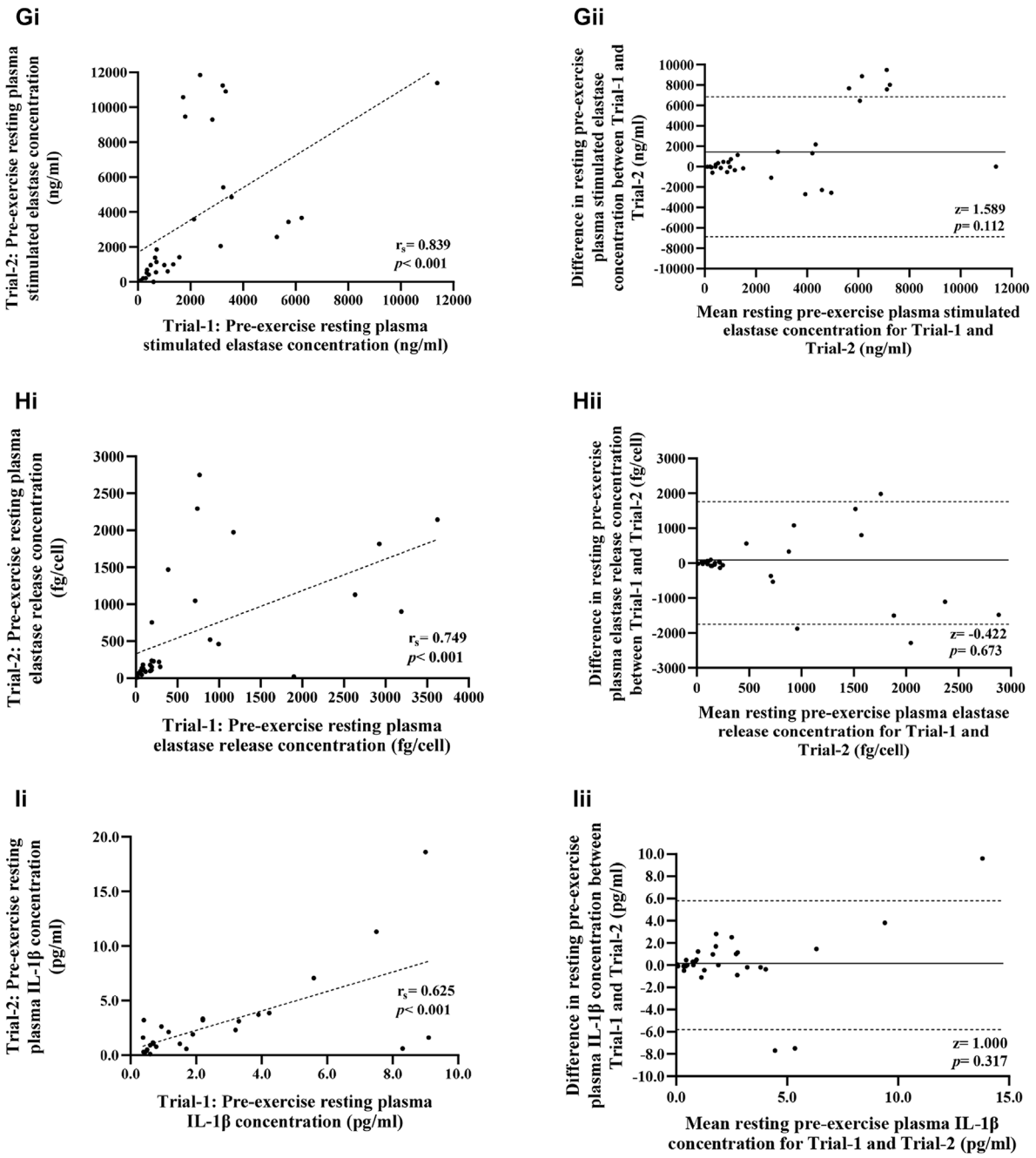

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concentration (r = 0.306, p = 0.027). There was no significant relationship between pre-exercise resting fecal and plasma total and differential concentrations of SCFA with the magnitude of change of plasma bacterial DNA from pre-to post-exercise ($p > 0.05$).

Discussion

The current study primarily aimed to comprehensively determine the test-retest reliability of selected biomarkers linked to EIGS at rest prior to exercise and in response to prolonged strenuous exercise. A secondary aim was to assess the association between luminal and systemic SCFA concentration with these variables. Firstly, at rest, no comparative test differences, with no to low Cohen's *d* effect size, and significant two-tailed correlation was observed between Trial-1 and Trial-2 for plasma concentrations of cortisol, I-FABP, sCD14, LBP, systemic inflammatory cytokines (i.e., IL-1β, TNF-α, IL-10, and IL-1ra), *in-vitro* bacterially-stimulated elastase, plus neutrophil counts, and fecal and plasma bacterial α-diversity. On the application of ICC, moderate to good

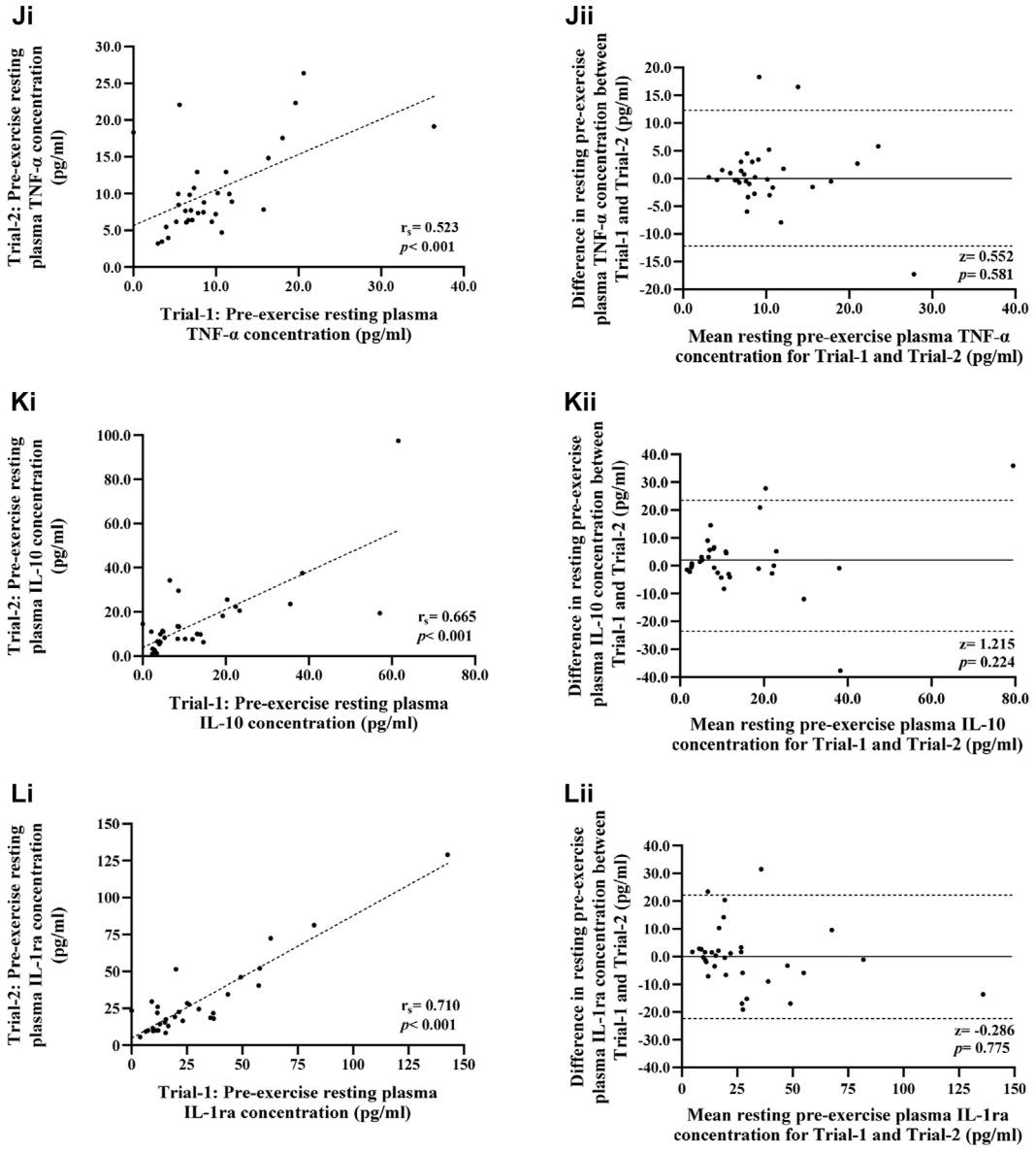

FIGURE 2
Correlation i) and Bland-Altman plot ii) comparing combined data from Trial-1 and Trial-2 for resting pre-exercise plasma cortisol **(A)**, I-FABP **(B)**, sCD14 **(C)**, and LBP **(D)** concentration; total leukocyte **(E)** and neutrophil counts **(F)**; total bacterially-stimulated elastase release **(G)** and elastase release per cell **(H)**; and plasma IL-1β **(I)**, TNF-α **(J)**, IL-10 **(K)**, and IL-1ra **(L)** concentration. Dotted line represents limits of agreement (±2 SD; 95% confidence interval), and the solid line represents mean bias between trials.

test-retest reliability was observed for plasma concentrations of cortisol, LBP, systemic inflammatory cytokines (i.e., IL-1β, TNF-α, IL-10, and IL-1ra), *in-vitro* bacterially-stimulated elastase, and fecal bacterial α-diversity. In response to exercise, 2 h of HIIT perturbed some biomarkers indicative of EIGS, including inducing bacteremia (i.e., quantity and diversity). No comparative test differences, with no to low Cohen's *d* effect size, and significant (or trend) two-tailed correlation were observed between Trial-1 and Trial-2 in response to exercise for plasma concentrations of cortisol, I-FABP, *in-vitro* bacterially-stimulated elastase, plus leukocyte and neutrophil counts, and plasma bacterial α-diversity. However, on the application of ICC, all markers were considered poor, except for *in-vitro* bacterially-stimulated elastase which was considered moderate for test-retest reliability. Secondly, to the best of our knowledge, this is the first study to directly measure the association between fecal and plasma SCFA concentrations with markers of EIGS. At rest, moderate test-retest reliability was observed for fecal and plasma SCFA concentrations. A novel finding was that pre-exercise plasma butyrate concentration was negatively associated with post-exercise plasma I-FABP concentration, indicative of intestinal epithelial injury. Taken together, the current data indicates: 1) 2 h of HIIT induced mild perturbations to markers indicative of EIGS, including bacteremia; 2) various EIGS biomarkers present good to excellent test-retest reliability at rest in response to rigorous pre-exercise

TABLE 4 Reliability indices of pre-exercise resting exercise-induced gastrointestinal syndrome (EIGS) biomarkers (A), plus fecal and plasma microbial α-diversity (B) and short chain fatty acid (SCFA) concentrations (C) between Trial-1 and Trial-2.

A. EIGS biomarkers	Intraclass correlation coefficient r [a]	95% CI	p	SEM[b]	MDC[c]
Cortisol (nMol/L)	0.87	0.75 to 0.93	**<.001**	134	370
IFABP (pg/ml)	0.21	−0.14 to 0.51	.199	469	1301
sCD14 (μg/ml)	0.38	0.06 to 0.64	**.010**	0.28	0.78
LBP (μg/ml)	0.76	0.56 to 0.88	**<.001**	3.9	10.7
Total leukocyte counts ($x10^9$/L)	0.33	−0.02 to 0.60	**.032**	1.83	5.07
Neutrophil counts ($x10^9$/L)	0.32	−0.04 to 0.59	**.038**	1.71	4.74
Total stimulated elastase (ng/ml)	0.44	0.12 to 0.68	**.004**	2843	7882
Elastase release per cell (fg/ml)	0.54	0.22 to 0.75	**.001**	800	2218
IL-1β (pg/ml)	0.64	0.39 to 0.81	**<.001**	2.28	6.31
TNF-α (pg/ml)	0.56	0.27 to 0.75	**<.001**	5.3	14.6
IL-10 (pg/ml)	0.73	0.53 to 0.86	**<.001**	10.2	28.2
IL-1ra (pg/ml)	0.92	0.84 to 0.96	**<.001**	9.5	26.2
Plasma bacterial DNA (ng/μl)	0.47	−0.03 to 0.79	**.035**	0.03	0.08
B. Microbial α-diversity					
Fecal SEI[d]					
Phyla	0.29	−0.17 to 0.67	.114	0.07	0.18
Family	0.56	0.08 to 0.83	**.014**	0.03	0.07
Genus	0.54	0.04 to 0.82	**.019**	0.03	0.08
Plasma SEI[d]					
Phyla	0.49	0.03 to 0.79	**.012**	0.05	0.13
Family	0.04	−0.52 to 0.54	.448	0.03	0.09
Genus	−0.16	−0.67 to 0.39	.712	0.04	0.04
C. SCFA					
Total fecal SCFA (μg/g)	0.53	0.17 to 0.77	**.003**	35.3	97.7
Fecal acetate (μg/g)	0.52	0.16 to 0.77	**.003**	26.6	73.7
Fecal propionate (μg/g)	0.44	0.05 to 0.72	**.016**	6.8	19.0
Fecal butyrate (μg/g)	0.45	0.04 to 0.73	**.017**	6.3	17.4
Fecal valeric (μg/g)	0.48	0.08 to 0.75	**.012**	1.1	2.9
Fecal caproic (μg/g)	0.56	0.20 to 0.79	**.003**	0.6	1.7
Total plasma SCFA (μg/L)	0.59	0.29 to 0.78	**<.001**	62.3	172.8
Plasma acetate (μg/L)	0.59	0.28 to 0.79	**<.001**	60.5	167.6
Plasma propionate (μg/L)	0.14	−0.02 to 0.46	.212	6.2	17.3
Plasma butyrate (μg/L)	0.63	0.37 to 0.79	**<.001**	2.9	7.9

[a]Intraclass correlation coefficient (ICC), whereby $r^a < 0.5$, $r^a = 0.5$-0.74, $r^a = 0.75$-0.9, and $r^a > 0.90$ for poor, moderate, good and excellent reliability respectively and 95% confidence intervals (CI).
[b]SEM (Standard Error of Measurement).
[c]MDC (Minimal Detectable Change).
[d]SEI (Shannon Equitability Index).

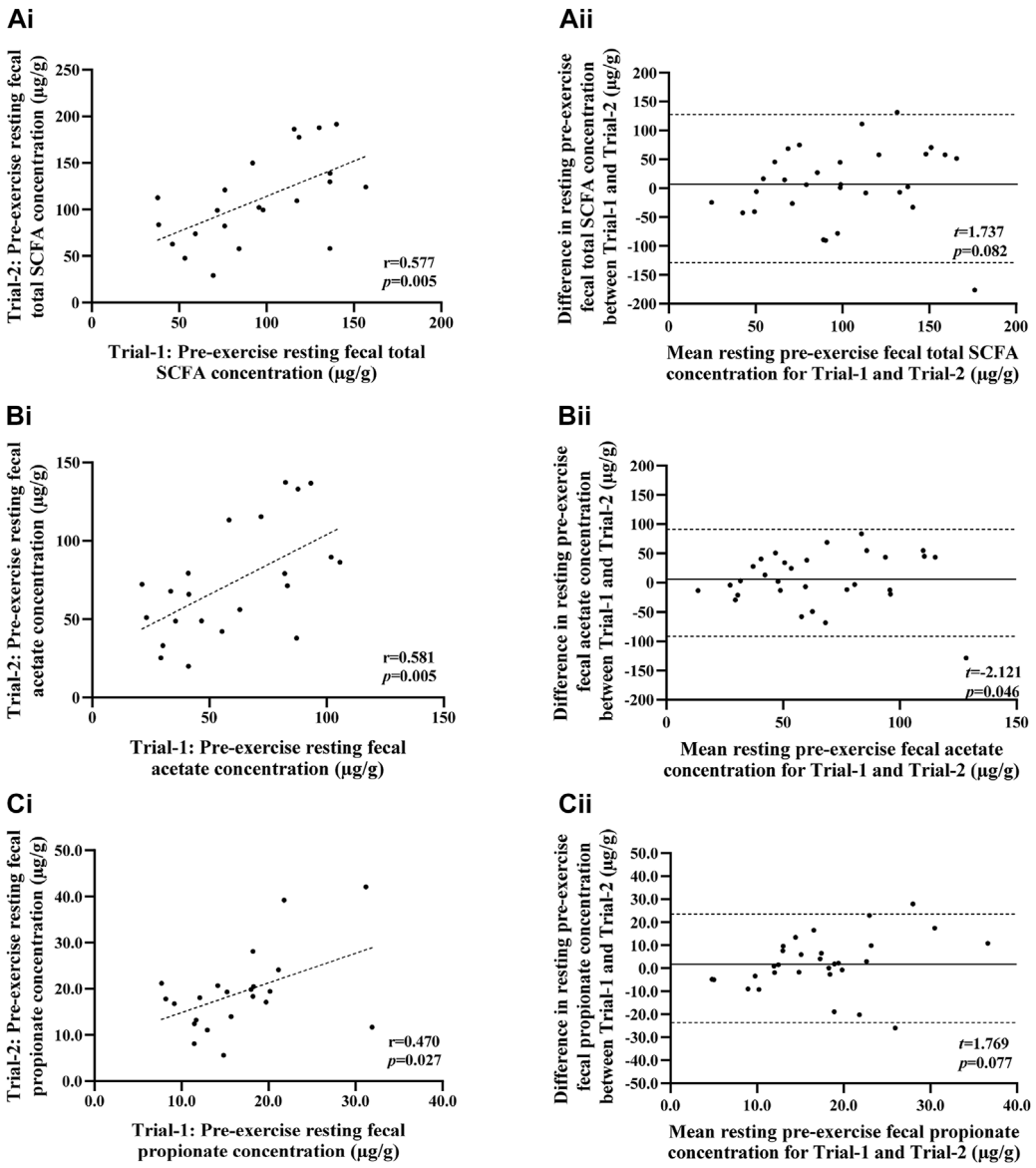

FIGURE 3
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experimental confounder control; 3) no EIGS biomarker presented adequate test-retest reliability in isolation in response to exercise (i.e., 2 h HIIT); and 4) with rigorous experimental control (e.g., standardised dietary provisions), pre-exercise fecal and plasma SCFA concentrations demonstrate an association with biomarkers of EIGS. These outcomes provide support for the need for greater exertional stress protocols and rigorous experimental control of potential confounding factors when studying EIGS. In addition, the findings provide support for the need to assess a cluster of EIGS biomarkers commonly used in exercise gastroenterology research, due to the ease of application and low-medium invasiveness, that assess the impact of exercise on gastrointestinal status and systemic responses linked to performance and clinical implications (Costa et al., 2022).

However, it is important to note that due to participant availability within the respective original studies, the washout period between test and re-test was not consistent between participants, varying between 5- to 14-days. This may constitute a limitation in reliability of the current meta-data, warranting caution when interpreting reliability of single EIGS biomarker. Nevertheless, while this study provides necessary insights into reliability of markers when using exertional-stress models that modestly perturb gastrointestinal and immune responses, further exploration is warranted when using exercise protocols known to provoke clinically relevant changes to all biomarkers of EIGS (≥2 h in heat or ≥3 h duration).

Biomarkers of gastrointestinal epithelial integrity (e.g., I-FABP), lumen originated bacterial endotoxin translocation (e.g., sCD14 and

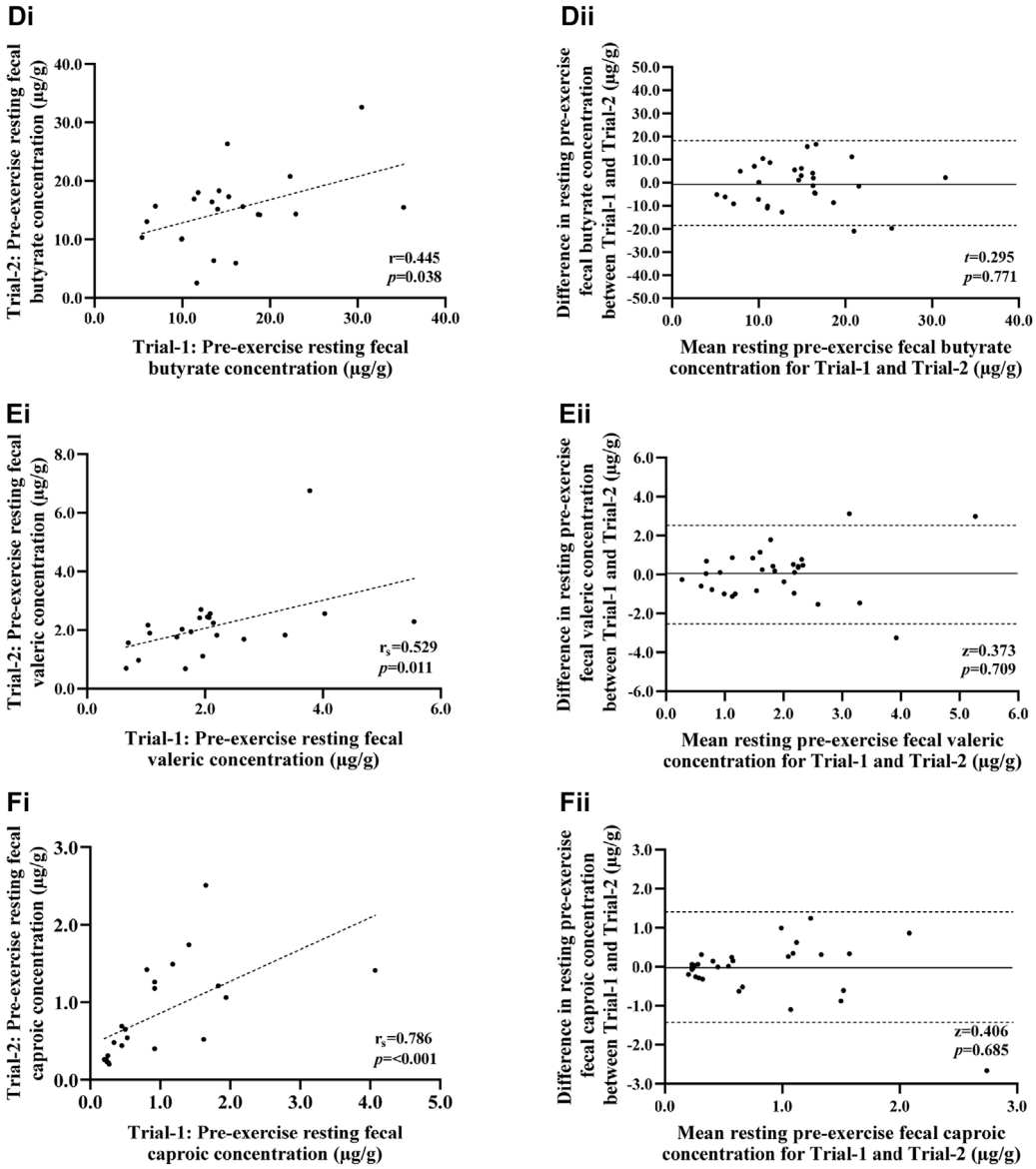

FIGURE 3
(continued)

LBP), and systemic immune responses (e.g., leukocyte counts, *in-vitro* neutrophil function, and systemic inflammatory cytokine profile) have widespread application in exercise-gastroenterology research and are critical in establishing the magnitude of EIGS and its degree of clinical significance. The current study provided a comprehensive reliability analysis, using paired *t*-tests (or non-parametric equivalents), Cohen's *d* effect size, two-tailed correlation, and intra-class correlation coefficients, on a multitude of biomarkers in application of adequate exertional-stress (i.e., 2 h HIIT). All resting pre-exercise biomarkers presented with small effect sizes ($d \leq 0.40$) and no differences between trials ($p > 0.05$). The most robust measures based on test-retest analysis were plasma concentrations of cortisol, LBP, and anti-inflammatory cytokines IL-10 and IL-1ra, which all exhibited good-to-excellent reliability (ICC) and strong correlations ($r = 0.665$). Plasma LBP concentration provides evidence of internal exposure to gram-negative bacterial endotoxin lipopolysaccharide, functioning as an indirect marker of luminal to systemic endotoxemia as a result of increased intestinal permeability (Costa et a., 2017b; Seethaler et al., 2021). Interestingly, a minimal effect size ($d = 0.08$), strong correlation ($r = 0.813$) and good ICC (ICC = 0.76) was associated with resting measures of plasma LBP concentration providing strong justification for its application as a primary biomarker of intestinal permeability and exercise associated endotoxemia. This notion is supported by a recent study that reported a strong correlation between resting plasma LBP concentration and the dual sugars lactulose/mannitol ratio, independent of age, BMI, and biological sex (Seethaler et al., 2021). The application of anti-inflammatory cytokines, IL-10 and IL-1ra, as principle biomarkers

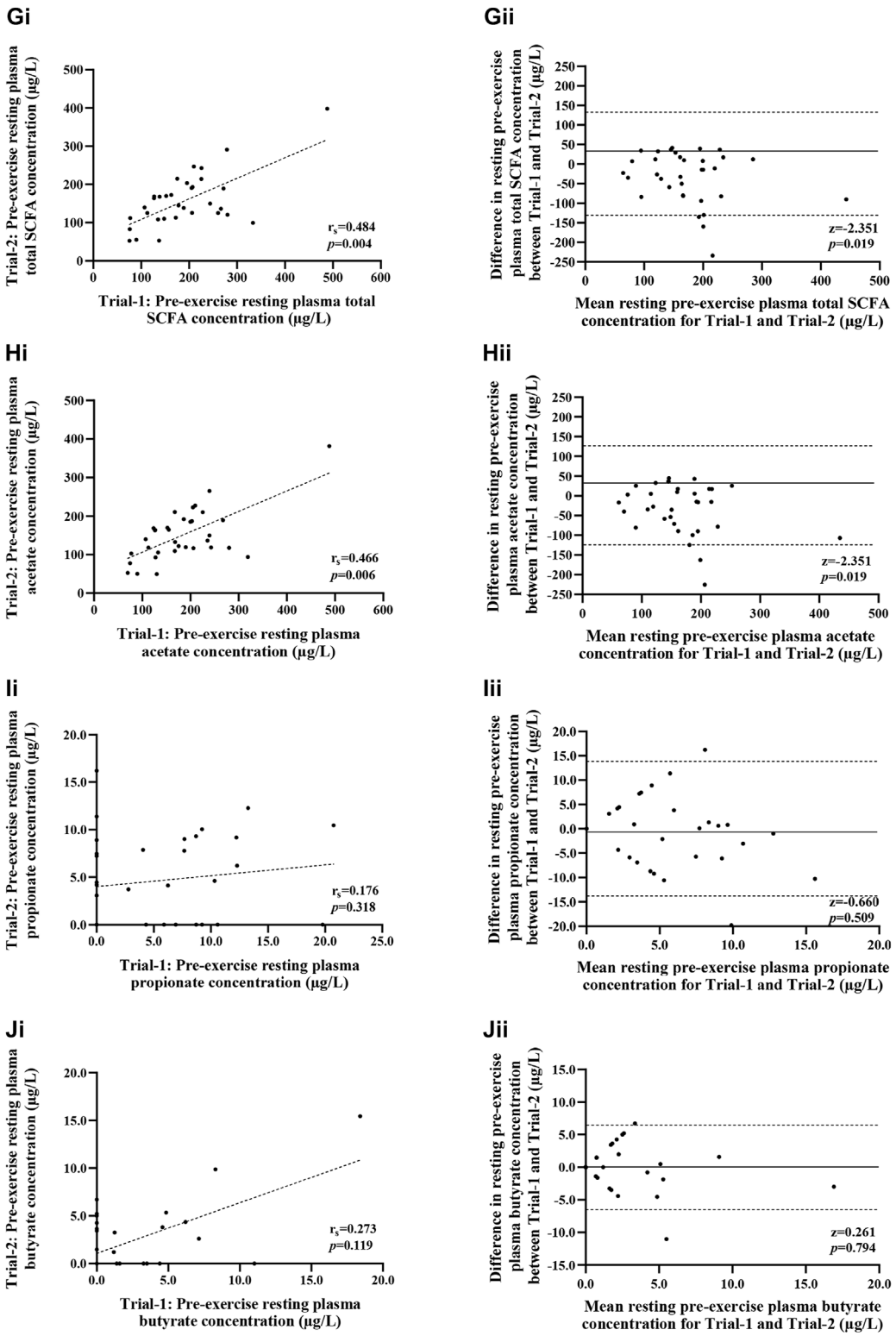

FIGURE 3
Correlation i) and Bland-Altman plot ii) comparing combined data from Trial-1 and Trial-2 for resting pre-exercise fecal total SCFA **(A)**, fecal acetate **(B)**, fecal propionate **(C)**, fecal butyrate **(D)**, fecal valeric **(E)**, fecal caproic **(F)**, plasma total SCFA **(G)**, plasma acetate **(H)**, plasma propionate **(I)** and plasma butyrate **(J)**. Dotted line represents limits of agreement (±2 SD; 95% confidence interval), and the solid line represents mean bias between trials.

for measuring the degree of exercise-induced systemic inflammatory response is also warranted, because of: 1) reliability outcomes, 2) consistent response sensitivity in comparison to other systemic inflammatory profile cytokines, 3) consistency in response to exertional and exertional-heat stress models, and 4) magnitude of response links to clinical outcomes (i.e., pro- to anti-inflammatory ratio) (Costa et al., 2022). Both cytokines presented large correlations and good-to-excellent ICCs (ICC = 0.73 and ICC = 0.92, respectively). Elevations in these biomarkers indicate a transient systemic pro-inflammatory response (i.e., increased IL-1β and TNF-α) has occurred, characteristic of pathogenic luminal translocation into systemic circulation, resulting in activation of systemic inflammatory and compensatory anti-inflammatory responses (Gill et al., 2015a; Gill et al., 2015b; Peake et al., 2015; Gill et al., 2016b; Costa et al., 2022). Based on combined ICC and correlation analysis, alternative variables of EIGS (i.e., *in-vitro* neutrophil function, neutrophil counts, and plasma concentrations I-FABP, sCD14, and pro-inflammatory cytokines IL-1β and TNF-α) displayed moderate test-retest reliability, and despite minimal effect sizes, it is recommended that these biomarkers are used in combination and/or in conjunction with more reliable measures. Leukocyte counts exhibited the poorest reliability with no significant correlation (r = 0.327, $p > 0.05$) and a poor ICC (0.33); therefore, continued use is only justified as a supportive biomarker of established immune measures with caution required in interpretation and reporting.

A novel and benchmark outcome of this study is the establishment of MDC of each resting biomarker (Table 4), stipulating reference cut-off values that are indicative of true physiological change. In context of the strenuous exercise applied in this study, despite significant changes in several biomarkers pre-to post-exercise, no marker increased beyond the associated MDC. This confirms 2 h of HIIT is not as potent a stimulus of EIGS, as seen with longer exercise duration and/or heat exposure during exercise (Snipe et al., 2017; Snipe and Costa, 2018a; Snipe and Costa, 2018b; Gaskell et al., 2021c; Gaskell et al., 2021d), and confirms the concerns highlighted in a recent methodological review by Costa et al. (2022). Of particular interest is the MDC associated with I-FABP, a measure applied consistently as a principle marker of gastrointestinal perturbations within exercise gastroenterology research. Previously a change of ≥1000 pg/ml from baseline was understood to reflect significant acute gastrointestinal integrity disturbances of clinical significance (Pelsers et al., 2005; Haas et al., 2009; Jekarl et al., 2015; Surbatovic et al., 2015; Al-Saffar et al., 2017; Linsalata et al., 2018; Martinez-Fierro et al., 2019; Power et al., 2021), seen consistently as a result of aggressive exercise protocols (2 h exertional heat-stress or ≥3 h duration) (Snipe and Costa, 2018a; Snipe and Costa, 2018b; Snipe & Costa, 2018b; Gaskell et al., 2019; Gaskell et al., 2021a). However, findings from this study reveal that in effect, a change of ≥1300 pg/ml, is necessary to confirm true physiological change as opposed to a change attributable to measurement error. Amidst the establishment of test-retest reliability of EIGS variables, MDC reference values function to support accurate translation of research findings into practice and safeguard against prospective misinterpretation and overstated conclusions that have been a component of exercise-gastroenterology research in the past (Snipe et al., 2018b).

In contrast with resting measures, test-retest reliability of exercise-associated magnitude response was highly variable, and while most magnitude response measures demonstrated no difference between trials (aside from plasma IL-10 concentration), and small effect sizes, correlation and ICC analysis revealed biomarkers to have either poor-to-moderate reliability (i.e., plasma cortisol and I-FABP concentration, leukocyte and neutrophil counts) or no reliability (plasma sCD14, LBP, IL-1β, TNF-α, and IL-1ra concentrations). *In-vitro* bacterially-stimulated elastase was the only marker that displayed good test-retest reliability (ICC = 0.72), with moderate-good correlation (r = 0.646). Although, a lower mean HR and RPE was reported in Trial-2 *vs.* Trial-1, likely due to a trial order effect, it did not appear to influence the overall physiological and thermal strain, which are the key aspects of physiological stress likely to impact the magnitude of EIGS biomarkers (Costa et al., 2017b; Costa R. J. S. et al., 2020; Costa et al., 2022). In view of the relatively modest impact of 2 h HIIT on gastrointestinal disturbances, poorer test-retest reliability is expected to be observed when applying more aggressive exercise-stress models (i.e., ≥2 h in heat), attributable to commonly observed increases in intra- and inter-variation between individuals. In addition, despite rigorous control of the experimental protocol in the current study, as per Costa et al. (2022), controlling for this variability evidently remains challenging. Accordingly, the SEM and MDC associated with magnitude of change response biomarkers were deemed irrelevant and not included in analysis, with use of MDC reference values related to resting measures recommended when verifying if a change from pre-to post-exercise is of clinical relevance.

Despite the widespread application of these biomarkers in exercise-gastroenterology research, only one other study has previously reported on reliability characteristics of EIGS biomarkers in relation to exercise-stress (Ogden et al., 2020a). Unfortunately, some limitations were associated with the study design, including not applying an exercise protocol sufficient to cause any substantial EIGS biomarker response (80 min walking protocol). This likely explains the small magnitude response in assessed biomarkers and inevitability of less response variability, and subsequent suggestion of adequate reliability of assessed markers. The experimental limitation deems post-exercise and magnitude response reliability analysis translationally limited, making it challenging to appraise in the context of the current findings. Furthermore, the reliability analysis was performed on a limited number of biomarkers (i.e., I-FABP, LBP, dual-sugar test, claudin-3, total 16S DNA concentrations and *Bacteriodes*/total bacterial DNA), with no systemic cytokines indicative of luminal pathogenic translocation of clinical relevance included, and some included markers recognised to provide erroneous outcomes and prone to misinterpretation (Costa et al., 2022). For example, *Bacteriodes*/total bacterial DNA, is a non-established measure that was used to quantify whole bacteria translocation from lumen to systemic circulation, and was reported to have poor-reliability (Ogden et al., 2020b). The current study expanded on the concept of whole bacteria lumen to systemic circulation translocation by measuring and reporting full plasma bacterial composition profile in addition to total bacterial DNA concentration profile in response to more substantial exercise stress. The findings support no Trial-1 *vs.*

Trial-2 differences in pre-exercise resting and post-exercise bacterial DNA concentration with no-to-low Cohen's *d* effect size, but no significant two-tail correlation and poor ICC outcomes. In addition, plasma bacteria α-diversity at phyla, family, and genus level presented similar outcomes for comparative tests and effect size, with no resting and exercise-induced magnitude response two-tail correlation and poor ICC outcomes. Surprisingly, regardless of methodological discrepancies, both studies displayed robust reliability for pre-exercise resting LBP measures (d = .07, r = 0.85 *vs.* d = .08, r = 0.813), but this did not translate to exercise-associated Δ magnitude response (d = .39, r = −0.16 *vs.* d = .11, r = 0.119). In contrast to findings from the current study, plasma I-FABP displayed strong reliability at rest (r = 0.750) (Ogden et al., 2020a), compared to weak test-retest correlations reported at the same time point in the current study (r = 0.411). ICC analysis further substantiated a weak test-retest reliability with an ICC of 0.21 pre-exercise. Contrasting findings between the two studies are not unexpected considering the differences in exercise protocols and control measures. It is expected that test-retest reliability of these biomarkers in response to exercise will further lessen when applying more potent exertional stress models attributable to the commonly observed intra- and inter-variation in gastrointestinal perturbations (i.e., the greater the exercise associated gastrointestinal perturbation, the higher the likelihood of individual variability) (Costa et al., 2022). To confirm or contrast this assumption, future reliability analysis is required in well-controlled studies using exercise models that mimick real-world exercise activities that consistently report incidence and greater severity of gastrointestinal issues (e.g., ≥2 h heat or ≥3 h, and/or ultra-endurance based activities).

Metabolic by-products of commensal bacteria (e.g., SCFA-acetate, butyrate and propionate) along the gastrointestinal tract are known to maintain intestinal epithelial integrity through regulation of tight junctions, enhancement of epithelial cell stability, and reduction in permeability In turn, there is a reduced risk of translocation of pathogenic agents within the intestinal lumen into circulation, attenuating local and systemic inflammatory responses (Sekirov et al., 2010; Canani et al., 2011; Gilert et al., 2018). In the current study, it was observed that increased concentrations of plasma butyrate pre-exercise, was associated with reduced exercise-associated perturbations to the intestinal epithelium, as evidenced by lower concentrations of plasma I-FABP post-exercise. In addition, higher fecal acetate concentrations pre-exercise was linked to higher plasma concentrations of pro-inflammatory cytokines, IL-1β and TNF-α; however, the total magnitude of response of IL-1β and TNF-α to the exercise protocol was insignificant and of no clinical relevance. Other plasma and fecal SCFA concentrations showed little to no association with EIGS biomarkers following 2 h HIIT. It is the authors understanding that the current study is the first to directly assess the association between SCFAs and markers of EIGS. While it is acknowledged that correlations do not infer an effect, it is not unexpected that butyrate may offer some protection against intestinal epithelial injury considering its positive physiological effects at intestinal level that have been observed consistently *in-vitro* and *in-vivo*, in both general and clinical populations (Mariadason et al., 1997; Canani et al., 2011; Clarke et al., 2011). More recent studies using human-exercise models have hypothesised the potential of SCFAs to attenuate EIGS. Bennett et al. (2020) observed that greater *a*-diversity and relative SCFA-producing commensal bacteria attenuates the degree of EIGS experienced by endurance-trained athletes exercising for 2 h in hot conditions. While not directly investigating SCFAs impact on gut barrier integrity, the authors theorised the increased SCFA production associated with higher relative abundance of commensal bacteria was responsible for reduced EIGS. Interpretation of findings from the current study should be done in the context of variable test-retest reliability of SCFA biomarkers ranging from poor-to-moderate for both fecal and plasma measures. The relatively low reliability may be indicative of the sensitivity of these measures to confounding factors and large inter- and intra-subject variability. Further exploration is warranted in order to identify these potential confounders of plasma and fecal SCFA levels, with special consideration to plasma butyrate given findings from the current study and the understanding of the role butyrate may have in maintaining epithelial cell integrity. It is suggested that future studies start by adopting an extended pre-trial dietary control protocol to help reduce some variability in these measures.

Bacterial endotoxin translocation is well-understood to be a key instigator of transient systemic inflammation during and following substantial exertional load (Costa et al., 2017b; Costa R. J. S. et al., 2020), but whole bacteria luminal to systemic translocation is a novel concept that warranted further exploration with scarce research to date and nothing comprehensive in the exercise research setting (Castillo et al., 2019; Costa et al., 2022; Villarroel et al., 2022). The current study is the first to comprehensively explore exercise-associated bacteremia (i.e., concentration and composition) using an exertional stress model reflective of real-life practices of endurance and team-sports athletes that have the potential to foster gastrointestinal symptoms (Steege et al., 2008; Engebretsen et al., 2013). Findings demonstrate that 2 h of HIIT results in a significant increase in plasma bacterial DNA concentration from pre-to post-exercise. Interpretation of bacterial presence in circulation warrants some caution however, due to the contamination risk associated with sample handling (i.e., sample collection, processing and analysis procedures), posing a challenge in distinguishing between true biological bacterial DNA in plasma and that related to contaminated consumables and samples (Nikkari et al., 2001). To minimise the risk of cross-contamination and including artefact values in data analysis, risk management procedures were employed as described in the methods section and only bacterial groups with a conservative ≥0.5% relative abundance, respective to the determination medium, were included for data analysis as per methods and proposed in previous studies (Bokulich et al., 2013; Lou et al., 2021). Plus, the included blank control samples (i.e., pyrogen and DNAse/RNAse from water) underwent the same processing procedures, yielding negative detectable outcomes. Furthermore, resting levels of

plasma bacterial DNA have significantly low biomass compared to that residing in the gastrointestinal tract, presenting a risk of DNA falling below the detectable limit. In the current study, while the total plasma DNA concentration of some participants pre-exercise was undetected (i.e., below the minimal detection level of <0.02 ng/μl), plasma DNA concentration was quantifiable for all participants post-exercise consistent across trials validating a true increase in total plasma DNA, likely with substantial attributions from whole bacteria lumen to circulating translocation. Lastly, the authors acknowledge that exercise-induced cellular damage and consequential increases in cellular free DNA can lead to misinterpretation of findings (Agassi et al., 2015), which have been overcome in the current study through determination of bacterial composition profile. Findings demonstrated significant changes to relative abundance of certain phyla, family and genus groups from pre-to post-exercise protocol, consistent across trials, providing further evidence of bacterial translocation. Preceding studies, albeit scarce, showed dissimilar pre- to post-exercise plasma bacterial profile outcomes, likely related to differing exertional stress models and measurement methodologies and data presentation (e.g., total bacterial s16 DNA to *Bacteroides* ratio) (March et al., 2019; Ogden et al., 2020a, 2020b). A novel finding in the current study was that *Proteobacteria* (phyla, family and genus), and not *Bacteroides* and *Firmicutes,* demonstrated the greatest translocation, creating significant shifts in alpha diversity (i.e., reduction in alpha diversity at phyla level associated with increased *Proteobacteria*). As a result, a focus on *Proteobacteria* as a measure of bacterial translocation is justified over *Bacteriodes*. In support of previous research of the healthy blood microbiome, *Proteobacteria* was the most dominant phyla, accounting for 63% of the bacterial composition pre-exercise (Castillo et al., 2019). Mechanistically, *Proteobacteria* and the healthy *vs*. disease state blood microbiome is still poorly understood. Previous studies, however, have consistently highlighted a correlation between gut dysbiosis (i.e., inflammation associated with IBD) and increased *Proteobacteria* in the gastrointestinal tract, with the notion it may play a pro-inflammatory role in disease states (Frank et al., 2007; Mukhopadhya et al., 2012; Matsuoka and Kanai, 2014; Rizzatti et al., 2017; Caruso et al., 2020). It is hypothesised that EIGS-associated transient inflammation of the gastrointestinal tract stimulates an increase in luminal *Proteobacteria* concentration and subsequent translocation into plasma, offering a potential explanation for the high relative abundance of *Proteobacteria* phyla, family and genus in circulation. Of interest would be post-exercise endoscopic findings that reflect acute transient changes occurring at the terminal ilium (i.e., where epithelial cell lining is thin and subject to damage), which would perhaps demonstrate increased *Proteobacteria* concentrations related to exercise-induced gut disturbances. Unfortunately, post-exercise fecal samples are limited due to bacterial DNA dominating the sigmoid colon, and not the critical point at the terminal ilium. To confirm and expand on findings from the current study, further research using sufficient exercise stress models reflective of real-life training and competition of endurance and/or ultra-endurance athletes that suffer from gastrointestinal issues during exercise is needed, with exploration in the implications of changes in bacteremia on Ex-GIS, performance and clinical outcomes.

Conclusion

The 2 h HIIT protocol was sufficient in inducing perturbations to certain biomarkers of EIGS, consistent across both trials. EIGS-associated disturbances also initiated luminal to systemic translocation of whole bacteria, evidenced by an increase in total DNA concentrations pre-to post-exercise and alterations in the blood microbiome profile. The potential impact of significant bacterial translocation during endurance exercise on Ex-GIS, performance and health still needs to be explored. Extensive test-retest analysis exposed that commonly used biomarkers of EIGS have variable reliability, ranging from poor-to-excellent. It is, therefore, strongly advised that a cluster of biomarkers is used and interpreted collectively when assessing EIGS, and/or general gastrointestinal integrity and systemic responses to exercise. The application of a limited number of measures risks erroneous interpretations due to large variability within and between individuals in response to exercise. A limitation to this study was the use of an exercise protocol that only moderately perturbed select EIGS biomarkers, making it challenging to accurately evaluate the reliability of markers when measuring magnitude of response. Of value would be repeating similar reliability analysis using more substantial exertional-stress models well-established in inducing significant changes to EIGS biomarkers, using the current minimal detectable change (MDC) as guidance. Lastly, a moderate-negative correlation was observed between pre-exercise plasma butyrate and concentrations of plasma I-FABP post-exercise, suggesting an association between higher levels of butyrate and reduced exercise-associated perturbations to the intestinal epithelium. Further research is warranted to confirm this relationship and investigate if SCFA concentrations have a role in attenuating the severity of EIGS.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra/PRJNA926792.

Ethics statement

The studies involving human participants were reviewed and approved by Monash University- Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

The authors' contributions are as follows: RC was the chief investigator of this research and contributed to the original strategy for further data analysis, in collaboration with PY. RC and IR, contributed towards development of the original experimental designs that led to the generation of data. PG and JM contributed to development and analysis of fecal and plasma samples to determine SCFA concentrations. RH, PY, and RC contributed toward the management and determination of fecal and plasma bacterial DNA and taxa. PY and ZD contributed towards the raw data analysis. PY and RC prepared the original draft manuscript. All authors contributed to the review and final preparation of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1063335/full#supplementary-material

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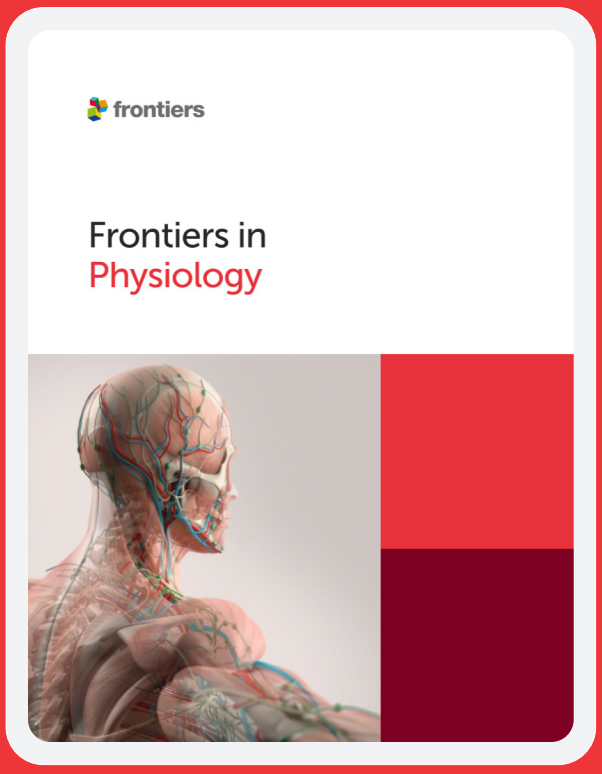

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